INVESTIGATIONS IN FISH CONTROL

77. Efficacy of 3-Trifluoromethyl-4-nitrophenol (TFM), 2′,5-Dichloro-4′-nitrosalicylanilide (Bayer 73), and a 98:2 Mixture as Lampricides in Laboratory Studies

78. Toxicity of the Molluscicide Bayer 73 and Residue Dynamics of Bayer 2353 in Aquatic Invertebrates

79. Accumulation, Elimination, and Biotransformation of the Lampricide 2′,5-Dichloro-4′-nitrosalicylanilide by *Chironomus tentans*

UNITED STATES DEPARTMENT OF THE INTERIOR
FISH AND WILDLIFE SERVICE

Investigations in Fish Control, published by the Fish and Wildlife Service, include reports on the results of work at the Service's Fish Control Laboratories at La Crosse, Wis., and Warm Springs, Ga., and reports of other studies related to that work. Though each report is regarded as a separate publication, several may be issued under a single cover, for economy. [See Investigations in Fish Control 47–50 (in one cover) for list of issues published prior to 1970.]

(Reports 41 through 43 are in one cover.)

41. Identification of MS-222 Residues in Selected Fish Tissues by Thin Layer Chromatography, by John L. Allen, Charles W. Luhning, and Paul D. Harman. 1970. 7 pp.
42. Dynamics of MS-222 in the Blood and Brain of Freshwater Fishes During Anesthesia, by Joseph B. Hunn. 1970. 8 pp.
43. Effect of MS-222 on Electrolyte and Water Content in the Brain of Rainbow Trout, by Wayne A. Willford. 1970. 7 pp.
44. A Review of Literature on TFM (3-trifluormethyl-4-nitrophenol) as a Lamprey Larvicide, by Rosalie A. Schnick. 1972. 31 pp.

(Reports 45 and 46 are in one cover.)

45. Residues of MS-222 in Northern Pike, Muskellunge, and Walleye, by John L. Allen, Charles W. Luhning, and Paul D. Harman. 1972. 8 pp.
46. Methods of Estimating the Half-Life of Biological Activity of Toxic Chemicals in Water, by Leif L. Marking. 1972. 9 pp.

(Reports 47 through 50 are in one cover.)

47. Preparation and Properties of Quinaldine Sulfate, an Improved Fish Anesthetic, by John L. Allen and Joe B. Sills. 1973. 7 pp.
48. Toxicity of Quinaldine Sulfate to Fish, by Leif L. Marking and Verdel K. Dawson. 1973. 8 pp.
49. The Efficacy of Quinaldine Sulfate as an Anesthetic for Freshwater Fish, by Philip A. Gilderhus, Bernard L. Berger, Joe B. Sills, and Paul D. Harman. 1973. 9 pp.
50. Residue of Quinaldine in Ten Species of Fish Following Anesthesia with Quinaldine Sulfate, by Joe B. Sills, John L. Allen, Paul D. Harman, and Charles W. Luhning. 1973. 9 pp.

(Reports 51 and 52 are in one cover.)

51. Methods for Simultaneous Determination and Identification of MS-222 and Metabolites in Fish Tissues, by Charles W. Luhning. 1973. 10 pp.
52. Residues of MS-222, Benzocaine, and Their Metabolites in Striped Bass Following Anesthesia, by Charles W. Luhning. 1973. 11 pp.

(Reports 53 through 55 are in one cover.)

53. Toxicity of Mixtures of Quinaldine Sulfate and MS-222 to Fish, by Verdel K. Dawson and Leif L. Marking. 1973. 11 pp.
54. The Efficacy of Quinaldine Sulfate:MS-222 Mixtures for the Anesthetization of Freshwater Fish, by Philip A. Gilderhus, Bernard L. Berger, Joe B. Sills, and Paul D. Harman. 1973. 9 pp.
55. Residues of Quinaldine and MS-222 in Fish Following Anesthesia with Mixtures of Quinaldine Sulfate:MS-222, by Joe B. Sills, John L. Allen, Paul D. Harman, and Charles W. Luhning. 1973. 12 pp.

(Reports 56 through 59 are in one cover.)

56. Toxicity of the Lampricide 3-Trifluoromethyl4nitrophenol (TFM) to 10 Species of Algae, by A. W. Maki, L. D. Geissel, and H. E. Johnson. 1975. 17 pp.
57. Actue Toxicities of 3-Trifluoromethyl-4-nitrophenol (TFM) and 2′,5-Dichloro-4′-nitrosalicylanilide (Bayer 73) to Larvae of the Midge *Chironomus tentans*, by J. A. Kawatski, M. M. Ledvina, and C. R. Hansen. 1975. 7 pp.
58. Acute Toxicity of the Lampricide 3-Trifluoromethyl-4-nitrophenol (TFM) to Nymphs of Mayflies (*Hexagenia* sp.), by C. R. Fremling. 1975. 8 pp.
59. Toxicity and Residue Dynamics of the Lampricide 3-Trifluoromethyl-4-nitrophenol (TFM) in Aquatic Invertebrates, by H. O. Sanders and D. F. Walsh. 1975. 9 pp.

NOTE: Use of trade names does not imply Government endorsement of commercial products.

INVESTIGATIONS IN FISH CONTROL

77. Efficacy of 3-Trifluoromethyl-4-nitrophenol (TFM), 2′,5-Dichloro-4′-nitrosalicylanilide (Bayer 73), and a 98:2 Mixture as Lampricides in Laboratory Studies

By Verdel K. Dawson, Kenneth B. Cumming, and Philip A. Gilderhus

78. Toxicity of the Molluscicide Bayer 73 and Residue Dynamics of Bayer 2353 in Aquatic Invertebrates

By Herman O. Sanders

79. Accumulation, Elimination, and Biotransformation of the Lampricide 2′,5-Dichloro-4′-nitrosalicylanilide by *Chironomus tentans*

By Joseph A. Kawatski and Ann E. Zittel

UNITED STATES DEPARTMENT OF THE INTERIOR
FISH AND WILDLIFE SERVICE
Washington, D.C. • 1977

Efficacy of 3-Trifluoromethyl-4-nitrophenol (TFM), 2′,5-Dichloro-4′-nitrosalicylanilide (Bayer 73), and a 98:2 Mixture as Lampricides in Laboratory Studies

by

Verdel K. Dawson, Kenneth B. Cumming, and Philip A. Gilderhus
Fish Control Laboratories, P.O. Box 862
La Crosse, Wisconsin 54601

Abstract

The lampricidal effects of 3-trifluoromethyl-4-nitrophenol (TFM), 2′,5-dichloro-4′-nitrosalicylanilide (Bayer 73), and a 98:2 mixture of the two (TFM:2B) were tested against larvae of the sea lamprey *(Petromyzon marinus)* under controlled laboratory conditions. The lampricides were tested in water at temperatures of 7, 12, and 17 C; total hardnesses of 44, 170, and 300 mg/l as $CaCO_3$; and pH's of 6.5, 7.5, and 8.5. Temperature had little influence on the toxicity of the lampricides, but the effect of Bayer 73 was slowed in cold water. Water hardness did not significantly influence the activity of the 98:2 mixture. The toxicities of TFM, Bayer 73, and TFM:2B were significantly reduced in water of high pH. Burrowed sea lamprey larvae were less vulnerable to TFM, Bayer 73, and TFM:2B than were free-swimming larvae. TFM and TFM:2B were selective for free-swimming lampreys over the nontarget organisms used for comparison, but the margin of safety for nontarget organisms over burrowed sea lampreys was narrow.

Although a number of methods have been used to control the parasitic sea lamprey *(Petromyzon marinus)* in the Great Lakes, the most widely used and most successful method has been application of chemical lampricides. Applegate et al. (1958) reported that 3-trifluoromethyl-4-nitrophenol (TFM) was selective against the sea lamprey, and in 1964 the compound was registered by the Pesticide Registration Division of the U.S. Department of Agriculture for limited use to control sea lamprey larvae in tributaries of the Great Lakes.

In 1963, 2′,5-dichloro-4′-nitrosalicylanilide (Bayer 73 or Bayluscide) was found to be extremely toxic to sea lampreys, and the addition of small amounts of Bayer 73 to TFM greatly reduced the amount of TFM required for effective treatment of lamprey populations (Howell et al. 1964). Because Bayer 73 is also very toxic to other fish and is virtually nonselective for lampreys over rainbow trout *(Salmo gairdneri)*, not more than 3% by weight can be added to TFM without losing the selective toxicity of TFM (Howell et al. 1964). Since 1963, mixtures of TFM and Bayer 73 have been used to control lampreys in tributaries of the Great Lakes by both the United States and Canadian governments (Hamilton 1974).

Use of the mixture has produced occasional fish kills that could not be predicted on the basis of pretreatment toxicity tests (U.S. Bureau of Commercial Fisheries 1968). These fish kills have been attributed to problems with formulation, application, water chemistry, or a combination of these factors (U.S. Bureau of Commercial Fisheries 1964, 1968; Smith 1966). Numerous tests on the lampricidal activity of the combination have been conducted in waters of various hardnesses, pH's, and temperatures. However, most of the studies were conducted in water from natural sources, where the presence of several variables and undetermined factors made it difficult to evaluate the influence of individual water characteristics (Erkkila 1964; Howell et al. 1964; U.S. Bureau of Commercial Fisheries 1964; Davis et al. 1965; Smith 1966). Recent studies supporting continued registration of the lampricide mixture have defined the influence of water chemistry on the toxicity to nontarget aquatic organisms (Kawatski et al. 1974; Kawatski et al. 1975; Bills and Marking 1976). Additional information on the efficacy of the mixture is needed to support its continued registration as a lampricide.

The purpose of the present study was to determine: (1) the toxicities of TFM, Bayer 73, and the TFM:2B mixture to sea lamprey larvae; (2) the individual and combined influences of temperature, water hardness, and pH on toxicity; (3) the toxicities to burrowed and exposed (free-swimming) lamprey larvae; and (4) the safety to nontarget organisms (selectivity).

Materials and Methods

Lamprey larvae collected from the Jordan River (Michigan) with electrofishing gear with anesthetized with MS-222 (100 mg/l) and sorted according to species and size. Sea lamprey larvae used as test organisms (average length, 8 cm; range, 5-10 cm) were held for at least 2 weeks before testing in troughs containing 12 C well water flowing over a 10-cm-deep sand substrate.

Field grade TFM (35.7% in dimethylformamide) was obtained from American Hoechst Corporation, and Bayer 73 (70% wettable powder) from Chemagro Corporation. Bayer 73 was applied at concentrations corresponding to 2% of the TFM concentrations, based on active ingredients of each, as recommended by Howell et al. (1964). The chemicals were tested simultaneously, both singly and in combination, at each temperature, hardness, and pH. The effect of combining the two chemicals was evaluated by the use of an additive index (Marking and Dawson 1975).

The toxicants were added to the test vessels 20 h after the introduction of lampreys. Ten lamprey larvae were exposed to each concentration in 15-liter glass jars according to the method of Lennon and Walker (1964). Test waters of different quality were produced by adding selected reconstituting salts to deionized water. The pH in the various tests was adjusted and maintained with chemical buffers, as suggested by Dawson et al. (1975). Water temperatures of 7, 12, and 17 C were controlled by water baths.

Dead larvae were counted and removed at 1, 3, 6, and 12 h, and daily thereafter, during the 96-h tests. Observations are reported at 12 h because that period approximates the average duration of chemical treatments of streams. LC_{50}'s, LC_{01}'s, LC_{99}'s, and 95% confidence intervals were computed according to the method of Litchfield and Wilcoxon (1949). The LC_{99}'s computed for lampreys statistically approximate the minimum concentrations needed for complete kills of the test organisms. Concentrations of both toxicants were reported on the basis of active ingredient. A P value of 0.05 was used to evaluate significance.

Because larvae usually live in burrows in the substrate of streams, the toxicity of TFM to burrowed lampreys, as well as lampreys confined without substrate, was determined. To minimize the effect of adsorption of the chemical by the substrate, we conducted tests in a flow-through test apparatus similar to that used by Marking et al. (1975). Burrowed and free-swimming sea lamprey larvae were held in separate screened compartments in the same test vessel. In addition to the sea lamprey larvae, we held rainbow trout, brook trout (*Salvelinus fontinalis*), and crayfish (*Procambarus* sp.) in the test chambers during the flow-through toxicity tests to accurately assess the selectivity of the lampricides. Selectivity was defined by a safety index (LC_{50} for brook trout divided by LC_{50} for sea lampreys) and a maximum safety index (LC_{01} for brook trout divided by LC_{99} for sea lampreys) similar to those employed by Marking (1967).

Results

Effect of Temperature

The 12-h LC_{99}'s of each compound—TFM, Bayer 73, or TFM:2B—against sea lamprey larvae differed little at different temperatures (7, 12, and 17 C; Table 1). Temperature did not significantly influence the toxicity of any of these compounds at exposure periods ranging from 3 to 96 h (Appendix 1), with one exception: the activity of Bayer 73 was slightly reduced in cold water (7 C) after 3 h of exposure, but not after 6 h or longer.

Effect of Water Hardness

Water hardnesses of 44, 170, and 300 mg/l as $CaCO_3$ did not significantly influence the toxicity of TFM, Bayer 73, or the mixture (Table 1), regardless of the period of exposure (Appendix 1).

Effect of pH

The toxicity of TFM was significantly decreased by increases in pH, as indicated by the 12-h LC_{99}'s (mg/l) of 0.660 at pH 6.5, 1.70 at 7.5, and 4.65 at 8.5 (Table 1). Although not as pronounced, the toxicity of Bayer 73 also was decreased at high pH's; the 12-h LC_{99}'s at pH 6.5, 7.5, and 8.5 were 0.0828, 0.145, and 0.165 mg/l, respectively (Table 1). Thus Bayer 73 was about twice as toxic and TFM about seven times as toxic at pH 6.5 as at 8.5. This decreased biological activity at high pH's was also evident in data reported as LC_{50}'s at all exposure periods (Appendix 1). As expected, the activity of the TFM:2B combination was also reduced at high pH (Table 1).

Effect of Substrate

The lampricide TFM was less toxic to sea lampreys burrowed in sand than to larvae confined without a substrate. A concentration of 5.63 mg/l killed all free-swimming larvae but none of the burrowed larvae in 6 h. In a concentration of 1.83 mg/l, all of the free-swimming larvae, but none of the burrowed larvae,

Table 1. *Toxicity (LC_{99} and 95% confidence interval)*[a] *of TFM (35.7%), Bayer 73 (70%), and TFM:2B (and additive indices for the mixture) to 8-cm sea lamprey larvae after 12 h of exposure in waters of selected temperatures, hardnesses, and pH's.*

Temp (°C)	Water hardness (mg/l as $CaCO_3$)	pH	Individual		Mixture		Additive index and range
			TFM	Bayer 73	TFM	Bayer 73	
7	44	7.5	1.60 1.24-2.07	0.0680 0.0407-0.114	1.14 0.694-1.87	0.0233 0.0142-0.0383	-0.0551 -1.45 to 1.17
12	44	7.5	1.70 1.11-2.59	0.145 0.0886-0.237	1.80 1.12-2.90	0.0367 0.0231-0.0582	-0.312 -2.27 to 0.887
17	44	7.5	1.20 0.835-1.72	0.120 0.0754-0.191	1.22 0.782-1.90	0.0249 0.0159-0.0391	-0.224 -1.79 to 0.859
12	170	7.5	1.59 1.01-2.51	0.125 0.0741-0.211	1.46 0.945-2.26	0.0298 0.0193-0.0459	-0.157 -1.86 to 1.14
12	300	7.5	1.58 1.00-2.49	0.108 0.0614-0.190	1.48 0.989-2.22	0.0302 0.0203-0.0449	-0.216 -1.95 to 0.984
12	44	6.5	0.660 0.387-1.13	0.0828 0.0487-0.141	0.425 0.277-0.652	0.00867 0.00565-0.0133	0.336 -0.958 to 2.51
12	44	8.5	4.65 3.02-7.15	0.165 0.0971-0.280	3.74 2.47-5.65	0.0763 0.0505-0.115	-0.267 -2.06 to 0.902

[a] Concentrations based on mg/l of active ingredient.

Table 2. *Toxicity (12-h LC_{99} for sea lampreys and 12-h LC_{01} for rainbow trout, brook trout, and crayfish, and 95% confidence interval)*[a] *of TFM (35.7%), Bayer 73 (70%), and TFM:2B (and the additive indices for the mixture) in flow-through toxicity tests in carbon filtered city water at 12 C.*

Species	Individual		Mixture		Additive index and range
	TFM	Bayer 73	TFM	Bayer 73	
Sea lamprey (burrowed)	5.39 3.80-7.64	0.280 0.249-0.314	12.5 7.51-20.8	0.255 0.153-0.425	-2.23 -6.18 to -0.470
Sea lamprey (free-swimming)	3.00 2.11-4.26	0.0920 0.0662-0.128	1.64 1.03-2.61	0.0335 0.0211-0.0533	0.0979 -1.04 to 1.46
Rainbow trout	3.95 3.43-4.55	0.0255 0.0228-0.0286	1.83 1.63-2.05	0.0374 0.0333-0.0420	-0.930 -1.44 to -0.523
Brook trout	4.00 3.48-4.60	0.0245 0.0218-0.0275	2.10 1.83-2.40	0.0428 0.0373-0.0491	-1.27 -1.94 to -0.754
Crayfish	8.20 7.06-9.53	>0.150[b]	>7.00	>0.143	—

[a] Concentrations based on mg/l of active ingredient.

[b] No mortality at highest concentration tested.

were dead after 12 h, and 20% of the burrowed larvae were still alive after 96 h. The 12-h LC_{99}'s (mg/l) for TFM were 5.39 against burrowed and 3.00 against free-swimming sea lamprey (Table 2).

In comparison with burrowed lampreys, free-swimming larvae were about three times more vulnerable to Bayer 73 and more than seven times more vulnerable to TFM:2B. The greater sensitivity of free-swimming larvae was evident for each chemical individually and in combination, at all exposure periods tested (Appendix 2).

Safety to Nontarget Organisms (Selectivity)

On the basis of the 12-h LC_{50}'s (Appendix 2), free-swimming (1.88 mg/l) and burrowed (3.39 mg/l) sea lamprey larvae were less resistant to TFM than were rainbow trout (6.10 mg/l), brook trout (6.00 mg/l), or crayfish (12.9 mg/l). However, to show ideal selectivity the lampricide should kill all lamprey larvae without harming nontarget organisms. A comparison of the LC_{99}'s for sea lampreys and the LC_{01}'s for nontarget species showed TFM to have a rather narrow margin of safety. The 12-h LC_{99}'s for burrowed (5.39 mg/l) and free-swimming (3.00 mg/l) sea lampreys were not significantly different from the 12-h LC_{01}'s for rainbow trout (3.95 mg/l) or brook trout (4.00 mg/l). The crayfish (8.20 mg/l), however, were significantly more resistant than free-swimming lampreys (Table 2).

The selectivity of the lampricides can also be represented by a safety index (Marking 1967), in which a value greater than 1.0 indicates selectivity for the target species, and a value less than 1.0 indicates that nontarget species could be harmed by concentrations effective against target species. The safety index (LC_{50} for trout divided by LC_{50} for lampreys) of 1.77 for TFM indicates selectivity for sea lampreys; however, the maximum safety index (LC_{01} for trout divided by LC_{99} for lampreys) of 0.742 indicates that some mortality of sensitive fishes could be expected (Table 3). On the basis of the safety indices, Bayer 73 (0.188) and the TFM:2B combination (0.952) did not demonstrate selectivity for burrowed sea lampreys in these tests. However, the maximum safety indices comparing exposures of free-swimming sea lampreys and brook trout to TFM (1.33) and TFM:2B (1.28) do show selectivity.

Discussion

The toxicities of TFM, computed on the basis of active ingredient, did not differ between the sea lamprey larvae used in the present study, which were collected in 1973 from the Jordan River (Michigan), and those used in a previous study (Dawson et al. 1975), which were collected in 1972 from the watershed of the Rifle River (Michigan).

Howell et al. (1964) interpreted the activity of a mixture of TFM and Bayer 73 as synergistic if all the sea lamprey larvae were killed at concentrations which were nontoxic when the chemicals were applied singly. Bills and Marking (1976) reported the toxicity of the mixture to be additive or less than additive (not synergistic) when it was tested against fish. The additive indices computed from our data support the conclusion that the toxicity of the mixture is additive or less than additive. However, Howell et al. (1964) and Smith et al. (1974) demonstrated an economic advantage of applying the mixture, i.e., the amount of TFM required to produce toxicosis was reduced while the selectivity was maintained.

Table 3. *Safety and maximum safety indices of TFM (35.7%), Bayer 73 (70%), and TFM:2B in flow-through toxicity tests against fingerling brook trout and burrowed sea lamprey larvae in carbon filtered city water at 12 C.*

Chemical	Sea lamprey		Brook trout		Safety index and range[a]	Maximum safety index and range[b]
	12-h LC_{50}	12-h LC_{99}	12-h LC_{50}	12-h LC_{01}		
TFM	3.39 2.87-4.01	5.39 3.80-7.64	6.00 5.21-6.91	4.00 3.48-4.60	1.77 1.29-2.41	0.742 0.455-1.21
Bayer 73	0.180 0.114-0.285	0.280 0.249-0.314	0.0338 0.0301-0.0379	0.0245 0.0218-0.0275	0.188 0.106-0.332	0.0875 0.0694-0.110
TFM:2B	3.15 2.27-4.37	12.5 7.51-20.8	3.00 2.63-3.42	2.10 1.83-2.40	0.952 0.602-1.51	0.168 0.0880-0.320

[a] LC_{50} for brook trout/LC_{50} for sea lamprey.

[b] LC_{01} for brook trout/LC_{99} for sea lamprey.

Although temperature changes have been blamed for incomplete kills during stream treatments (U.S. Bureau of Commercial Fisheries 1958; Smith and King 1970), laboratory studies have indicated that temperature has little effect on the toxicity of TFM (U.S. Bureau of Commercial Fisheries 1960; Applegate et al. 1961; Dawson et al. 1975). However, Applegate et al. (1961) reported that the rate of death slowed as the temperature decreased and that the selectivity against lampreys increased as the temperature dropped to near freezing.

Lowering the temperature has been reported to reduce the activity of Bayer 73 (Strufe and Gönnert 1962; Tibbles 1967). Our study indicated reduced activity of this compound in cold water after 3 h of exposure, but not after longer exposures. Apparently the effective contact time is extended at low temperatures. Generally, the influence of temperature on the lampricides is insignificant when compared with influences of other factors.

The reduced activity of TFM at high pH's presumably results from an increased ionization of the molecule (pKa = 6.07; Applegate et al. 1961). The un-ionized form of certain molecules is lipid soluble, and therefore more easily transported across the gills of fish (Sills and Allen 1971). The activity of Bayer 73 was slightly reduced at higher pH's. This reduction, which is consistent with results from previous studies (Gillett and Bruaux 1962; Marking and Hogan 1967; Farringer 1972), may result from ionization of the molecule at higher pH's. Meredith (1971) reported some loss of activity of Bayer 73 at pH's below 7, due to precipitation. We did not observe this phenomenon, probably because of the extremely low concentrations used in the toxicity tests.

We found that free-swimming lampreys were more vulnerable than burrowed lampreys to TFM, Bayer 73, and TFM:2B. Possibly the free-swimming lampreys are more excited and have a faster rate of metabolism and uptake than the burrowed lampreys. Also, the burrowed lampreys may be somewhat protected from exposure to the lampricides in the water. Field use concentrations of the lampricides for each stream are routinely determined in on-site toxicity tests against free-swimming lampreys. Results of these tests could indicate treatment concentrations which are insufficient to eliminate all burrowed lampreys. Our results do not support those of Applegate et al. (1958), who reported that concentrations of TFM lethal to all larval lampreys were essentially the same in jar tests and in treatments of a simulated stream.

A comparison of the LC_{99}'s for sea lampreys and the LC_{01}'s for nontarget species in our tests showed TFM to have a narrow margin of safety (working range). However, Howell and Marquette (1962) showed that the working range (minimum lethal to maximum allowable concentration) varied from time to time in a particular stream and that optimum conditions and time for stream treatment could be determined by conducting a number of bioassays in a stream.

Conclusions

1. The TFM:2B combination was effective for controlling sea lamprey larvae.
2. Temperature had little influence on the toxicity of TFM or TFM:2B to lampreys.
3. The rate of action of Bayer 73 was only slightly reduced at low temperatures.
4. Water hardness did not significantly influence the activity of the TFM:2B combination.
5. The toxicities of TFM, Bayer 73, and TFM:2B were significantly reduced in water of high pH.
6. Burrowed sea lamprey larvae were less vulnerable than free-swimming sea lamprey larvae to TFM, Bayer 73, and TFM:2B.
7. TFM and TFM:2B are selective for free-swimming sea lamprey larvae, but the margin of safety for sensitive nontarget organisms over burrowed sea lampreys is comparatively narrow.

References

Applegate, V. C., J. H. Howell, J. W. Moffett, B. G. H. Johnson, and M. A. Smith. 1961. Use of 3-trifluoromethyl-4-nitrophenol as a selective sea lamprey larvicide. Great Lakes Fish. Comm., Tech. Rep. 1. 35 pp.

Applegate, V. C., J. H. Howell, and M. A. Smith. 1958. Use of mononitrophenols containing halogens as selective sea lamprey larvicides. Science 127(3294):336–338.

Bills, T. D., and L. L. Marking. 1976. Toxicity of 3-trifluoromethyl-4-nitrophenol (TFM), 2′,5-dichloro-4′-nitrosalicylanilide (Bayer 73), and a 98:2 mixture to fingerlings of seven fish species and to eggs and fry of coho salmon. U.S. Fish Wildl. Serv. Invest. Fish Control 69. 9 pp.

Dodge. 1965. Comparison of bio-assay results using TFM and TFM plus Bayer 73, 1964. Appendix 12, pages 67–69 *in* Great Lakes Fishery Commission, Report of annual meeting, Ann Arbor, Michigan, June 22–24, 1965.

Dawson, V. K., K. B. Cumming, and P. A. Gilderhus. 1975. Laboratory efficacy of 3-trifluoromethyl-4-nitrophenol (TFM) as a lampricide. U.S. Fish Wildl. Serv. Invest. Fish Control 63. 13 pp.

Erkkila, L. F. 1964. Lamprey control and research in the United States. Pages 30–41 *in* Great Lakes Fishery Commission annual report for the year 1963. Ann Arbor, Michigan.

Farringer, J. E. 1972. The determination of the acute toxicity of rotenone and Bayer 73 to selected aquatic organisms. M.S. Thesis. University of Wisconsin, La Crosse, 32 pp.

Gillett, J., and P. Bruaux. 1962. Laboratory and field testing of Bayluscide (Bayer 73). Pflanzenschutz-Nachr. 15:70-74.

Hamilton, S. E. 1974. A review of the literature on the use of TFM-Bayluscide in fisheries. U.S. Fish Wildl. Serv. Lit. Rev. 74-03. NTIS [Natl. Tech. Inf. Serv.] No. PB-235 442/AS. 39 pp.

Howell, J. H., E. L. King, Jr., A. J. Smith, and L. H. Hanson. 1964. Synergism of 5,2'-dichloro-4'-nitrosalicylanilide and 3-trifluormethyl-4-nitrophenol in a selective lamprey larvicide. Great Lakes Fish. Comm., Tech. Rep. 8. 21 pp.

Howell, J. H., and W. M. Marquette. 1962. Use of mobile bioassay equipment in the chemical control of sea lamprey. U.S. Fish Wildl. Serv. Spec. Sci. Rep. Fish. 418. 9 pp.

Kawatski, J. A., V. K. Dawson, and M. L. Reuvers. 1974. Effect of TFM and Bayer 73 on *in vivo* oxygen consumption of the aquatic midge *Chironomus tentans*. Trans. Am. Fish. Soc. 103(3):551-556.

Kawatski, J. A., M. M. Ledvina, and C. R. Hansen, Jr. 1975. Acute toxicities of 3-trifluoromethyl-4-nitrophenol (TFM) and 2',5-dichloro-4'-nitrosalicylanilide (Bayer 73) to larvae of the midge *Chironomus tentans*. U.S. Fish Wildl. Serv. Invest. Fish Control 57. 7 pp.

Lennon, R. E., and C. R. Walker. 1964. Laboratories and methods for screening fish-control chemicals. U.S. Fish Wildl. Serv. Invest. Fish Control 1 (Circ. 185). 15 pp.

Litchfield, J. T., Jr., and F. Wilcoxon. 1949. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther. 99(2):99-113.

Marking, L. L. 1967. Toxicity of MS-222 to selected fishes. U.S. Fish Wildl. Serv. Invest. Fish Control 12. 10 pp.

Marking, L. L., T. D. Bills, and J. H. Chandler, Jr. 1975. Toxicity of the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) to nontarget fish in flow-through tests. U.S. Fish Wildl. Serv. Invest. Fish Control 61. 9 pp.

Marking, L. L., and V. K. Dawson. 1975. Method for assessment of toxicity or efficacy of mixtures of chemicals. U.S. Fish Wildl. Serv. Invest. Fish Control 67. 8 pp.

Marking, L. L., and J. W. Hogan. 1967. Toxicity of Bayer 73 to fish. U.S. Fish Wildl. Serv. Invest. Fish Control 19 (Resour. Publ. 36). 13 pp.

Meredith, R. 1971. The dependence on pH of the molluscicidal activity of the ethanolamine salt of 5,2'-dichloro-4'-nitrosalicylic anilide in solution in 10° artificial hard water. WHO [World Health Organ.] SCHISTO/71.12-WHO/VCB/71.297. 11 pp.

Sills, J. B., and J. L. Allen. 1971. The influence of pH on the efficacy and residues of quinaldine. Trans. Am. Fish. Soc. 100(3):544-545.

Smith, B. R. 1966. Lamprey control and research in the United States. Pages 31-45 *in* Great Lakes Fishery Commission annual report for the year 1964. Ann Arbor, Michigan.

Smith, B. R., and E. L. King, Jr. 1970. Lamprey control in the United States. Appendix C, pages 29-40 *in* Great Lakes Fishery Commission annual report for the year 1969. Ann Arbor, Michigan.

Smith, B. R., J. J. Tibbles, and B. G. H. Johnson. 1974. Control of the sea lamprey *(Petromyzon marinus)* in Lake Superior, 1953-70. Great Lakes Fish. Comm., Tech. Rep. 26. 60 pp.

Strufe, R., and R. Gönnert. 1962. Comparative studies on the influence of environmental factors upon the efficiency of Bayluscide. Pflanzenschutz-Nachr. 15:50-70.

Tibbles, J. J. 1967. Annual report of the Department of Fisheries of Canada to the Great Lakes Fishery Commission. Appendix 4, pages 1-111 *in* Great Lakes Fishery Commission, Report of annual meeting, Ann Arbor, Michigan, June 20-22, 1967.

U.S. Bureau of Commercial Fisheries. 1958. Lamprey control and research in the United States. Pages 34-50 *in* Great Lakes Fishery Commission annual report for the year 1958. Ann Arbor, Michigan.

U.S. Bureau of Commercial Fisheries. 1960. Sea lamprey control and research in the United States in 1960. Appendix 4, pages 1-9 *in* Great Lakes Fishery Commission, Report of annual meeting, Cleveland, Ohio, December 1-2, 1960.

U.S. Bureau of Commercial Fisheries. 1964. Progress report on sea lamprey control. Appendix 5, pages 1-7 *in* Great Lakes Fishery Commission, Report of annual meeting, Ann Arbor, Michigan, June 17-18, 1964.

U.S. Bureau of Commercial Fisheries. 1968. Sea lamprey program, 1967. Appendix 4, pages 1-32 *in* Great Lakes Fishery Commission, Report of annual meeting, Toronto, Ontario, June 18-20, 1968.

Appendix 1. *Toxicity (LC_{50} and 95% confidence interval)*[a] *of TFM (35.7%), Bayer 73 (70%), and TFM:2B (and additive indices for the mixture) against 8-cm larval sea lampreys in static toxicity test waters of selected temperatures, hardnesses*[b]*, and pH's.*

Exposure time (h)	Individual		Mixture		Additive index and range
	TFM	Bayer 73	TFM	Bayer 73	
Water temp 7 C, hardness 44, pH 7.5					
3	2.60 1.97-3.43	0.0970 0.0765-0.123	1.05 0.814-1.35	0.0214 0.0166-0.0275	0.601 -0.0448 to 1.69
6	1.40 1.13-1.73	0.0550 0.0393-0.0771	1.00 0.745-1.34	0.0204 0.0152-0.0273	-0.0852 -0.881 to 0.592
12	0.800 0.743-0.861	0.0370 0.0266-0.0515	0.490 0.358-0.671	0.0100 0.00730-0.0137	0.133 -0.418 to 0.794
24	0.770 0.637-0.931	0.0276 0.0208-0.0366	0.455 0.347-0.597	0.00928 0.00708-0.0122	0.0786 -0.524 to 0.766
48	0.510 0.394-0.660	0.0220 0.0158-0.0306	0.455 0.347-0.597	0.00928 0.00708-0.0122	-0.314 -1.29 to 0.321
96	0.345 0.246-0.484	0.0220 0.0158-0.0306	0.440 0.345-0.561	0.00898 0.00704-0.0114	-0.684 -2.00 to 0.0606
Water temp 12 C, hardness 44, pH 7.5					
3	>1.6[c]	0.0500 0.0340-0.0740	1.00 0.787-1.27	0.0204 0.0161-0.0259	>-0.0330
6	1.66 1.11-2.50	0.0500 0.0340-0.0740	1.00 0.787-1.27	0.0204 0.0161-0.0259	-0.0104 -0.912 to 0.876
12	0.900 0.708-1.15	0.0450 0.0330-0.0608	0.820 0.610-1.10	0.0164 0.0124-0.0225	-0.276 -1.24 to 0.357
24	0.730 0.612-0.870	0.0450 0.0330-0.0608	0.540 0.407-0.717	0.0110 0.00830-0.0146	0.0161 -0.614 to 0.655
48	0.730 0.612-0.870	0.0450 0.0330-0.0608	0.450 0.347-0.584	0.00918 0.00708-0.0119	0.219 -0.315 to 0.941
96	0.730 0.612-0.870	0.0400 0.0268-0.0598	0.375 0.255-0.551	0.00765 0.00520-0.0112	0.419 -0.318 to 1.63
Water temp 17 C, hardness 44, pH 7.5					
3	1.20 0.880-1.64	0.045 0.0333-0.0608	0.833 0.621-1.12	0.0170 0.0127-0.0228	-0.0719 -0.962 to 0.700
6	0.850 0.687-1.05	0.0450 0.0330-0.0608	0.485 0.351-0.670	0.00990 0.00720-0.0137	0.265 -0.390 to 1.21
12	0.740 0.618-0.885	0.0410 0.0309-0.0540	0.455 0.350-0.591	0.00928 0.00710-0.0121	0.189 -0.348 to 0.898
24	0.560 0.452-0.693	0.0410 0.0309-0.0540	0.455 0.350-0.591	0.00928 0.00715-0.0121	-0.0388 -0.699 to 0.571
48	0.508 0.423-0.610	0.0410 0.0309-0.0540	0.455 0.350-0.591	0.00928 0.00715-0.0121	-0.122 -0.789 to 0.416
96	0.496 0.415-0.593	0.0410 0.0309-0.0540	0.455 0.350-0.591	0.00928 0.00715-0.0121	-0.144 -0.816 to 0.384

Appendix 1.—Continued

Exposure time (h)	Individual		Mixture		Additive index and range
	TFM	Bayer 73	TFM	Bayer 73	
Water temp 12 C, hardness 170, pH 7.5					
3	1.69 1.33-2.15	0.0550 0.0416-0.0726	1.04 0.710-1.52	0.0212 0.0145-0.0310	0.00280 -0.892 to 0.887
6	1.25 0.907-1.72	0.0380 0.0259-0.0550	0.630 0.466-0.852	0.0129 0.00950-0.0174	0.186 -0.612 to 1.26
12	0.770 0.586-1.01	0.0350 0.0249-0.0491	0.560 0.435-0.721	0.0114 0.00888-0.0174	-0.0530 -0.929 to 0.637
24	0.625 0.488-0.802	0.0350 0.0249-0.0491	0.560 0.435-0.721	0.0114 0.00888-0.0147	-0.222 -1.07 to 0.382
48	0.625 0.488-0.802	0.0350 0.0249-0.0491	0.560 0.435-0.721	0.0114 0.00888-0.0147	-0.222 -1.07 to 0.382
96	0.625 0.488-0.802	0.0350 0.0249-0.0491	0.560 0.435-0.721	0.0114 0.00888-0.0147	-0.222 -1.07 to 0.382
Water temp 12 C, hardness 300, pH 7.5					
3	2.62 1.98-3.46	0.900 0.142-5.71	1.25 0.946-1.65	0.0255 0.0193-0.0340	0.979 -0.0724 to 2.61
6	1.54 1.18-2.02	0.0440 0.0371-0.0603	0.860 0.651-1.14	0.0175 0.0133-0.0232	0.0458 -0.591 to 0.840
12	0.765 0.582-1.01	0.0390 0.0266-0.0571	0.560 0.449-0.699	0.0114 0.00920-0.0143	-0.0243 -0.739 to 0.645
24	0.765 0.582-1.01	0.0390 0.0266-0.0571	0.560 0.449-0.699	0.0114 0.00920-0.0143	-0.0243 -0.739 to 0.645
48	0.710 0.530-0.951	0.0390 0.0266-0.0571	0.560 0.449-0.699	0.0114 0.00920-0.0143	-0.0810 -0.856 to 0.579
96	0.710 0.530-0.951	0.0390 0.0266-0.0571	0.560 0.449-0.699	0.0114 0.00920-0.0143	-0.0810 -0.856 to 0.579
Water temp 12 C, hardness 44, pH 6.5					
3	0.450 0.301-0.673	0.0480 0.0360-0.0640	0.381 0.308-0.472	0.00777 0.00628-0.00960	-0.00850 -0.835 to 0.799
6	0.300 0.224-0.401	0.0450 0.0337-0.0602	0.232 0.182-0.295	0.00473 0.00371-0.00602	0.138 -0.496 to 0.940
12	0.172 0.121-0.245	0.0330 0.0233-0.0467	0.225 0.176-0.288	0.00459 0.00359-0.00588	-0.447 -1.63 to 0.257
24	0.172 0.121-0.245	0.0310 0.0227-0.0423	0.170 0.121-0.239	0.00350 0.00250-0.00490	-0.101 -1.19 to 0.808
48	0.172 0.121-0.245	0.0300 0.0222-0.0406	0.170 0.121-0.239	0.00350 0.00250-0.00490	-0.105 -1.20 to 0.800
96	0.172 0.121-0.245	0.0300 0.0222-0.0406	0.170 0.121-0.239	0.00350 0.00250-0.00490	-0.105 -1.20 to 0.800

Appendix 1.—Continued

Exposure time (h)	Individual		Mixture		Additive index and range
	TFM	Bayer 73	TFM	Bayer 73	
Water temp 12 C, hardness 44, pH 8.5					
3	>4.00	0.120 0.0855-0.168	3.60 1.91-6.76	0.0734 0.0390-0.138	>-0.512
6	2.80 2.26-3.47	0.0810 0.0541-0.121	1.26 1.00-1.59	0.0257 0.0204-0.0324	0.303 -0.300 to 1.19
12	2.37 1.85-3.04	0.0660 0.0466-0.0930	1.26 1.00-1.59	0.0257 0.0204-0.0324	0.0857 -0.553 to 0.821
24	1.40 1.13-1.74	0.0440 0.0310-0.0620	1.26 1.00-1.59	0.0257 0.0204-0.0324	-0.484 -1.45 to 0.104
48	1.30 0.957-1.77	0.0440 0.0310-0.0620	0.580 0.453-0.743	0.0118 0.00920-0.0152	0.400 -0.267 to 1.47
96	1.30 0.957-1.77	0.0390 0.0266-0.0570	0.580 0.453-0.743	0.0118 0.00920-0.0152	0.336 -0.348 to 1.39

[a] Concentrations based on mg/l of active ingredient.

[b] Water hardness = mg/l as $CaCO_3$.

[c] No mortality at highest concentration tested.

Appendix 2. *Toxicity (LC_{50} and 95% confidence interval)*[a] *of TFM (35.7%), Bayer 73 (70%), and TFM:2B (and additive indices for the mixture) in flow-through toxicity tests against several aquatic organisms in carbon filtered city water at 12 C.*

Organism and exposure time (h)	Individual		Mixture		Additive index and range
	TFM	Bayer 73	TFM	Bayer 73	
Sea lamprey (burrowed)					
3	>11.9[b]	>0.15	>7.80	>0.159	—
6	9.40 6.80-13.0	>0.15	7.80 5.32-11.4	0.159 0.109-0.233	>-0.890
12	3.39 2.87-4.01	0.180 0.114-0.285	3.15 2.27-4.37	0.0643 0.0463-0.0891	-0.286 -1.30 to 0.373
24	1.68 1.45-1.95	0.180 0.114-0.285	2.35 1.87-2.95	0.0479 0.0381-0.0602	-0.665 -1.56 to -0.0927
48	1.68 1.45-1.95	0.180 0.114-0.285	2.35 1.87-2.95	0.0479 0.0381-0.0602	-0.665 -1.56 to -0.0927
72	1.68 1.45-1.95	0.129 0.0995-0.167	2.35 1.87-2.95	0.0479 0.0381-0.0602	-0.770 -1.64 to -0.187
96	1.68 1.45-1.95	0.129 0.0995-0.167	2.35 1.87-2.95	0.0479 0.0381-0.0602	-0.770 -1.64 to -0.187

Appendix 2.—Continued

Organism and exposure time (h)	Individual		Mixture		Additive index and range
	TFM	Bayer 73	TFM	Bayer 73	
Sea lamprey (free-swimming)					
3	16.6 12.2-22.5	>0.0800	>7.00	>0.140	—
6	3.60 2.98-4.35	>0.0800	2.31 2.10-2.54	0.0471 0.0427-0.0518	>-0.230
12	1.88 1.59-2.23	0.0625 0.0540-0.0724	0.760 0.573-1.01	0.0155 0.0117-0.0206	0.533 -0.0222 to 1.39
24	<1.48[c]	0.0350 0.0254-0.0482	0.760 0.573-1.01	0.0155 0.0117-0.0206	<0.0456
48	<1.48	0.0335 0.0275-0.0409	0.700 0.544-0.900	0.0143 0.0111-0.0184	<0.111
72	<1.48	0.0335 0.0275-0.0409	0.700 0.544-0.900	0.0143 0.0111-0.0184	<0.111
96	<1.48	0.0335 0.0275-0.0409	0.700 0.544-0.900	0.0143 0.0111-0.0184	<0.111
Brook trout					
3	9.65 8.41-11.1	>0.0800	8.10 6.29-10.4	0.165 0.128-0.212	>-1.71
6	6.00 4.98-7.23	0.0880 0.0696-0.111	3.00 2.63-3.42	0.0612 0.0537-0.0698	-0.195 -0.690 to 0.180
12	6.00 5.21-6.91	0.0338 0.0301-0.0399	3.00 2.63-3.42	0.0612 0.0537-0.0698	-1.31 -1.98 to -0.797
24	6.00 5.21-6.91	0.0338 0.0300-0.0380	3.00 2.63-3.42	0.0612 0.0537-0.0698	-1.31 -1.98 to -0.794
48	5.95 5.18-6.83	0.0338 0.0300-0.0380	3.00 2.63-3.42	0.0612 0.0537-0.0698	-1.31 -1.99 to -0.798
72	5.95 5.18-6.83	0.0338 0.0300-0.0380	3.00 2.63-3.42	0.0612 0.0537-0.0698	-1.31 -1.99 to -0.798
96	5.95 5.18-6.83	0.0338 0.0300-0.0380	3.00 2.63-3.42	0.0612 0.0537-0.0698	-1.31 -1.99 to -0.798
Rainbow trout					
3	16.8 12.3-22.9	0.0720 0.0604-0.0858	4.10 3.57-4.72	0.0836 0.0728-0.0963	-0.405 -0.978 to -0.00438
6	6.40 5.67-7.23	0.0415 0.0351-0.0490	3.45 2.74-4.34	0.0704 0.0559-0.0885	-1.24 -2.29 to -0.520
12	6.10 5.19-7.18	0.0353 0.0315-0.0396	2.47 2.20-2.77	0.0504 0.0449-0.0565	-0.833 -1.33 to -0.440
24	6.10 5.19-7.18	0.0345 0.0309-0.0386	2.47 2.20-2.77	0.0504 0.0449-0.0565	-0.866 -1.36 to -0.470
48	6.10 5.19-7.18	0.0179 0.0145-0.0221	2.47 2.20-2.77	0.0504 0.0449-0.0565	-2.22 -3.43 to -1.34
72	6.10 5.19-7.18	0.0179 0.0145-0.0221	2.47 2.20-2.77	0.0504 0.0449-0.0565	-2.22 -3.43 to -1.34
96	6.10 5.19-7.18	0.0179 0.0145-0.0221	2.47 2.20-2.77	0.0504 0.0449-0.0565	-2.22 -3.43 to -1.34

Appendix 2.—Continued

Organism and exposure time (h)	Individual		Mixture		Additive index and range
	TFM	Bayer 73	TFM	Bayer 73	
Crayfish					
3	>16.5	>0.15	>7.00	>0.140	—
6	16.5 12.2-22.4	>0.15	>7.00	>0.140	—
12	12.9 11.0-15.1	>0.15	>7.00	>0.140	—
24	12.9 11.0-15.1	>0.15	>7.00	>0.140	—
48	12.6 10.5-15.2	>0.15	>7.00	>0.140	—
72	12.6 10.5-15.2	>0.15	>7.00	>0.140	—
96	12.6 10.5-15.2	>0.15	>7.00	>0.140	—

[a] Concentrations based on mg/l of active ingredient.

[b] No mortality at highest concentration tested.

[c] Total mortality at lowest concentration tested.

Toxicity of the Molluscicide Bayer 73 and Residue Dynamics of Bayer 2353 in Aquatic Invertebrates

by

Herman O. Sanders
Fish-Pesticide Research Laboratory, Route 1
Columbia, Missouri 65201

Abstract

The molluscicide Bayer 73 (2-aminoethanol salt of 2',5-dichloro-4'-nitrosalicylanilide), a chemical used as a synergist in conjunction with 3-trifluoromethyl-4-nitrophenol (TFM) to control the sea lamprey *(Petromyzon marinus)* in tributaries of the Great Lakes, was tested against five species of crustaceans and two species of aquatic insects: daphnids *(Daphnia magna)*, aquatic sowbugs *(Asellus brevicaudus)*, scuds *(Gammarus pseudolimnaeus)*, glass shrimp *(Palaemonetes kadiakensis)*, crayfish *(Orconectes nais)*, damselfly nymphs *(Ischnura verticalis)*, and midge larvae *(Chironomus plumosus)*. The acute toxicities ranged from a 48-h EC_{50} (median effective concentration causing immobilization) of 0.2 mg/l for daphnids to a 48-h LC_{50} (concentration causing 50% mortality) of 25 mg/l for crayfish. In daphnids exposed continuously to Bayer 73, reproduction was not impaired at concentrations of 0.018 and 0.032 mg/l, but was significantly ($P<0.01$) reduced at concentrations of 0.056, 0.10, and 0.18 mg/l. Exposure to Bayer concentrations of 0.56, 1.0, and 1.8 mg/l significantly ($P<0.01$) reduced emergency of midges. All organisms exposed to Bayer 2353 (chlorosalicylic acid ring UL ^{14}C) accumulated radioactive residues in 24 h that ranged from 4 to 80 times (based on wet weight of whole organism) the water concentration of 1.2 ± 0.2 μg/l. Scuds and midge larvae eliminated 90% of the residues in 48 h.

The molluscicide Bayer 73 (2-aminoethanol salt of 2',5-dichloro-4'-nitrosalicylanilide), which is sold commercially as Bayluscide, is especially toxic to freshwater snails (Chemagro Corporation 1970). It has been used in Africa, southeastern Asia, and portions of South America to control several species of snails that are intermediate hosts of organisms causing schistosomiasis in man (Gönnert 1962). Bayer 73 is effective as an ovicide against snail eggs (Gillet and Bruaux 1962), as a herbicide to control the tropical water fern, *Salvinia aurticulata* (Wild and Mitchell 1970), and as a piscicide for controlling fish populations (Brynildson 1970). This molluscicide is also extremely toxic to larvae of the sea lamprey, *Petromyzon marinus* (Howell et al. 1964), and has been used by the U.S. Fish and Wildlife Service and the Canadian Department of Environment for sampling lamprey populations in tributaries of the upper Great Lakes. It is also used as a synergist with the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) for lamprey control (Howell et al. 1964).

Authorization for lampricidal uses of TFM and the TFM-Bayer 73 mixture was jeopardized when the U.S. Department of Agriculture, Pesticide Regulation Division, gave notice on 13 May 1970 that registration of TFM would be cancelled unless tolerances were obtained in fish and water. The U.S. Fish and Wildlife Service was granted an extension from the then new regulatory agency, the U.S. Environmental Protection Agency (EPA), on 22 February 1971, to obtain additional data on methodology for application, and toxicology of, TFM, Bayer 73, and their combination. Submissions for an Amended Registration and Petition for Exemption from Tolerance for TFM were filed with EPA in February 1976.

Laboratory and field studies have shown that a 0.1-mg/l concentration of Bayer 73, which is effective for controlling lamprey larvae (Howell et al. 1964; Smith 1967), could have an adverse effect on nontarget aquatic organisms, such as planarians, tubificids, and daphnids (Hunn 1973), mollusks (Gönnert 1962), and fishes (Marking and Hogan 1967). However, aquatic invertebrates with a hard exoskeleton, such as ostracods (Kawatski 1973), aquatic sowbugs, crayfish, dragonflies, and dobsonflies (Hunn 1973), and stonefly naiads (Sanders and Cope 1968) were not severely affected by exposure to Bayer 73. Meyer and Howell (1975) reported that nymphs of burrowing

mayflies (*Hexagenia* sp.) exposed to Bayer 73 in Lake Huron water at 12 C were 160 times more resistant to this chemical than were larval lampreys.

Although the effects of TFM on aquatic invertebrates have been documented (Smith 1967; Chandler and Marking 1975; Fremling 1975; Kawatski et al. 1975; Sanders and Walsh 1975), a recent review of the literature (Hamilton 1974) indicated a lack of information for evaluating the safety of Bayer 73 to aquatic invertebrates. The objectives of the study were to determine the acute toxicities of Bayer 73 to aquatic invertebrates in static tests and to determine the effect of continuous exposure of Bayer 73 on reproduction in daphnids *(Daphnia magna)* and emergence of midges *(Chironomus plumosus)*. In addition, the accumulation of ^{14}C-Bayer 2353 from water by seven aquatic invertebrates was determined.

Materials and Methods

Test animals included five species of crustaceans and two species of aquatic insects: early instar and mature daphnids; mature aquatic sowbugs *(Asellus brevicaudus)*; mature scud *(Gammarus pseudolimnaeus)*; mature glass shrimp *(Palaemonetes kadiakensis)*; juvenile crayfish *(Orconectes nais)*; early instar damselfly nymphs *(Ischnura verticalis)*; and first and early fourth instars of midge larvae. Daphnids, scuds, and midge larvae were from laboratory cultures and the other invertebrates were collected from streams or ponds near Columbia, Missouri. All organisms were acclimated to laboratory conditions by rearing or holding them in the dilution water at the test temperature. A combination of Duro-test and wide spectrum Grow-lux bulbs provided light for the cultures and all tests. The light cycle was controlled for a 16-h photoperiod.

The water used for cultures and all experiments was from a deep well; it had a pH of 7.4 and a total hardness of 270 mg/l as $CaCO_3$. Crayfish and midge larvae were exposed at 22 ± 1 C and all other organisms at 18 ± 1 C.

The Bayer 73 (Chemagro Corp., Lot No. 8059410) was supplied by the Fish Control Laboratory, LaCrosse, Wisconsin, as a wettable powder containing 70% 2-aminoethanol salt of 2′,5-dichloro-4′-nitrosalicylanilide. Concentrations were based on active ingredient.

Acute toxicity tests were conducted under static conditions; methods used were those recommended for standardized laboratory toxicity tests (Committee on Methods for Toxicity Tests with Aquatic Organisms 1975). Flow-through tests were performed in a system modeled after Mount and Brungs (1967). The method of Litchfield and Wilcoxon (1949) was used to estimate the LC_{50}'s (concentrations causing 50% mortality) or EC_{50}'s (median effective concentrations causing immobilization) and 95% confidence intervals. In flow-through tests, the incipient LC_{50} or lethal threshold concentration (Sprague 1969) was determined when the asymptote had been reached in the toxicity curve. This value was determined when the mortality in each aquarium in any 5-day period dropped to 10% of the original number of animals.

Reproductive studies were conducted with daphnids in a flow-through system, with a chemical delivery apparatus designed by Chandler et al. (1974). Ten first-instar daphnids up to 24 h old were placed in duplicate exposure vessels that contained 1 liter of water. Thus, 20 daphnids per group were exposed continuously through a complete life cycle (21 days) to concentrations of 0 (the control), 0.018, 0.032, 0.056, 0.10, and 0.18 mg/l of Bayer 73. The test solutions and controls contained 0.1 ml/l of ethanol. Test organisms were fed a suspension of yeast in sufficient amounts to support a stable population. Reproductive success was assessed by counting the offspring produced in each concentration and the control after the parent daphnids had been exposed for 21 days. The mean number of young produced per adult was determined by averaging the number of young produced in replicate tests. Data were analyzed by analysis of variance, and significant differences among treatments were determined by a multiple means comparison test (least significant difference, Snedecor and Cochran 1974).

Use of the rearing technique described by Biever (1965) for the colonization of chironomid larvae maintained a continuously reproducing population of midges. Before the experiments were begun, eggs collected from a rearing unit were hatched in a stender dish. One hundred first-instar larvae (1.5 mm long and up to 24 h old) were transferred into exposure vessels that had been previously prepared by adding 13 g of washed sand to 1 liter of water. During the test, larvae were fed a commercial dog food called Dog Kisses (Biever 1965).

A flow-through system as described in the daphnid studies was used to determine the effects of Bayer 73 on larval survival, pupation, and emergency of midges. Cast pupal skins at the water surface in test containers were counted and removed daily to determine emergence. The test was terminated at 30 days, when 80–90% of the control larvae had emerged. The effects of Bayer 73 on emergence were analyzed by analysis of variance on the arcsin transformation for proportions (angle = $\arcsin\sqrt{\text{percentage}}$) followed by a least significant difference test (Snedecor and Cochran 1974).

The radioactive Bayer 2353 (chlorosalicylic acid ring UL ^{14}C) used in this study was prepared by the

American Radiochemical Corporation, Sanford, Florida, and was obtained through the Fish Control Laboratory, LaCrosse, Wisconsin. The specific activity of 10.0 mCi/mM given for this labeled compound was confirmed by gas chromatographic analysis of the stock solutions.

The concentration used in the accumulation studies (1.2 ± 0.2 μg/l) was selected partly on the basis of the acute toxicity determined for the most sensitive species exposed. Johnson and Schoettger (1975) suggested that a concentration between 1/10 and 1/1000 of the EC_{50}, depending on the slope of the toxicity curve, be selected as the test concentration. On the basis of use pattern, field evaluations, and toxicity of the chemical, a factor of 0.01 was applied to the lowest confidence limit of the EC_{50} for *D. magna*.

Accumulation studies were conducted in a flow-through system that included the chemical delivery apparatus designed by Chandler et al. (1974). Exposure vessels were 2-liter glass aquaria containing 1 liter of well water. Stock solutions of the ^{14}C-Bayer 2353 were prepared in water and further diluted in the flow-through system. Organisms were not fed during the 5-day exposure.

Invertebrate samples were taken in triplicate, weighed, and prepared directly for radiometric analysis by homogenizing the whole organism. The homogenate was obtained by adding 6 ml of Triton X-100:toluene (2:3 v/v) emulsifier to each sample before grinding it (Johnson et al. 1971). The concentration of labeled compound in water was monitored radiometrically by taking triplicate 1-ml samples entering the exposure chambers and then adding 14 ml of Triton:toluene-fluor mixture. The radioactivity of the samples was measured with a Beckman 200-L liquid scintillation counter. All samples were corrected for quench and counted until a statistical counting error of 5% or less had been reached. Residue values and accumulation factors (residue concentration in organism/residue concentration in exposure water) were computed on a whole-body, wet weight basis.

Elimination of ^{14}C-Bayer 2353 residues in several of the invertebrates was measured by exposing the organisms to the compound until a plateau concentration was reached. The organisms were then transferred to clean flowing water and analyzed at 8, 24, and 48 h to measure the decline in whole-body residues.

A method proposed by Mount and Stephan (1967) for establishing acceptable toxicant limits for aquatic organisms under continuous exposure conditions was used to calculate an application factor for determining a toxicant concentration that has no adverse effect on reproduction in daphnids or life cycle alterations in chironomids. The application factor consists of the laboratory determined maximum acceptable toxicant concentration (MATC) that has no effect on the test animals during chronic exposure, divided by the 48-h EC_{50}.

Results

Toxicity

The acute toxicities of Bayer 73 varied greatly among aquatic invertebrates, and ranged from a 48-h EC_{50} of 0.2 mg/l for daphnids to a 48-h LC_{50} of 25 mg/l for crayfish (Table 1). The invertebrates with a soft integument (daphnids and midge larvae) were more susceptible than those with a highly chitinized exoskeleton (scud, glass shrimp, and crayfish).

Flow-through tests indicated that the compound was not toxic to early instar crayfish in a concentration of 50 mg/l for 24 h. However, continuous exposure produced toxic effects, and at 4 days the LC_{50} was 35 mg/l. Toxicity reached an asymptote at 10 days and a time-independent LC_{50} of 16 mg/l was estimated. The toxicity to scuds was not substantially affected by continuous exposure, and 24-h LC_{50}'s were similar in static and flow-through tests.

Daphnid Reproduction

Continuous exposure of daphnids through a complete life cycle (21 days) to 0.056, 0.10, or 0.18 mg/l of Bayer 73 in a flow-through system

Table 1. *Acute toxicity of Bayer 73 to five species of aquatic invertebrates in static tests.*

Organism and stage	LC_{50} (mg/l) and 95% confidence limits[a]	
	24 h	96 h
Daphnid, 1st instar	—	(0.2)[b] 0.14-0.27
Scud, mature	(5.6) 4.7-6.7	(2.4) 1.8-3.1
Glass shrimp, mature	(19) 12-29	(10) 7-15
Crayfish, juvenile	(32) 23-45	(25) 19-33
Midge, 4th instar larva	(2.1) 1.6-2.9	1.5[b] 1.1-2.2

[a] Toxicities are expressed in terms of EC_{50} for daphnid and midge larva and LC_{50} for the other organisms.

[b] 48-h values.

Table 2. *Survival and reproduction of* Daphnia magna *after a 21-day exposure to Bayer 73 at 18 ± 1 C.*

Concentration (mg/l)	Percent survival of adults[a]	Total young produced per surviving adult
0.0	95	1,586
0.018	95	1,545
0.032	85	1,553
0.056	90	726[b]
0.10	85	244[b]
0.18	45	101[b]

[a] Twenty young exposed at each concentration.

[b] Significantly different from controls ($P<0.01$), $n = 2$.

significantly reduced ($P<0.01$) reproduction (Table 2). Survival of the adult daphnids at the termination of the tests was 85-95% in all Bayer 73 concentrations and controls, except at 0.18 mg/l, in which adult survival was 45%. The number of young produced per parent daphnid ranged from 5 at 0.18 mg/l to 77 at concentrations of 0.032 and 0.018 mg/l. The mean number of young produced in the controls was 79.

The MATC for daphnids was estimated to be between 0.032 and 0.056 mg/l. The application factor (MATC/EC_{50}) was between 0.16 and 0.28 (Table 3).

Midge Emergence

Emergence of adult midges was significantly reduced ($P<0.01$) after 30 days exposure to Bayer 73 at concentrations of 0.56, 1.0, and 1.8 mg/l (Table 4). Emergence was significantly delayed ($P<0.05$) in the 0.32 mg/l concentration at 15 days; however, at 30 days the emergence time was similar to that in the controls. The pupal stage seems to be the most sensitive stage in the midge life cycle; some pupal mortality was noted in control chambers. The highest pupal mortality was in the 1.8-mg/l concentration; only 33% of the organisms emerged.

Table 3. *Ranges of application factors and safe concentrations for Bayer 73, continuous exposure, for* Daphnia magna *and the midge* Chironomus plumosus.

Organism	48 h EC_{50} (mg/l)[a]	MATC[b] (mg/1)	Application factor[c]
Daphnid	0.2 (0.14-0.27)	0.032-0.056	0.16-0.28
Midge larva	1.5 (1.1-2.2)	0.32-0.56	0.21-0.36

[a] Values in parentheses are 95% confidence limits of the EC_{50}.

[b] Highest concentration producing no observed effect and the lowest concentration in which an effect was observed.

[c] Limits of the maximum acceptable toxicant concentration (MATC), divided by 48-h EC_{50}.

The MATC for midges was estimated to be between 0.32 and 0.56 mg/l and the application factor was between 0.21 and 0.36 (Table 3).

Accumulation and Elimination

The accumulation of ^{14}C-Bayer 2353 was relatively low in the seven species of aquatic invertebrates, ranging from 4 to 80 times the water concentration of 1.2 ± 0.2 μg/l (Table 5). Accumulation of radioactive residues was greater in soft-bodied invertebrates (daphnids and midge larvae) than in organisms with a hard exoskeleton (glass shrimp and crayfish). Most invertebrates accumulated maximum residues

Table 4. *Cumulative percentages of midges* (Chironomus plumosus) *that emerged after continuous exposure of the larvae to different concentrations of Bayer 73 at 22 ± 1 C.*

Days of exposure	Bayer 73 concentration (mg/l)					
	0 (Control)	0.1	0.32	0.56	1.0	1.8
15	10	5	3[a]	4[a]	3[a]	0[a]
20	57	49	47	34[b]	15[b]	5[b]
25	84	79	78	45[b]	24[b]	27[b]
30	87	85	86	47[b]	53[b]	33[b]

[a] Significantly different from controls ($P<0.05$).

[b] Significantly different from controls ($P<0.01$).

Table 5. *Whole-body residues of ^{14}C-Bayer 2353 accumulated from water by seven aquatic invertebrates.*

Organism and stage	Water concentration (μg/l and SE)	Whole-body residues (μg/kg) and accumulation factors[a]			
		24 h	48 h	72 h	120 h
Daphnid, 1st instar	1.4 (0.02)	75 (4) [53]	78 (2) [56]	80 (2) [57]	75 (3) [53]
Aquatic sowbug, mature	1.1 (0.08)	25 (4) [23]	32 (2) [29]	30 (1) [27]	28 (1) [25]
Scud, mature	1.2 (0.25)	80 (4) [67]	88 (2) [73]	83 (4) [69]	85 (2) [71]
Glass shrimp, mature	1.0 (0.09)	4 (2) [4]	6 (2) [6]	9 (1) [9]	9 (1) [9]
Crayfish, juvenile	1.0 (0.17)	4 (2) [4]	8 (2) [8]	9 (1) [9]	11 (1) [11]
Damselfly, mature nymph	1.2 (0.10)	8 (2) [7]	12 (1) [10]	10 (1) [8]	8 (2) [7]
Midge, 4th instar larva	1.1 (0.03)	87 (7) [80]	90 (5) [82]	89 (2) [81]	80 (6) [73]

[a] Residue values are means of three samples (SE in parentheses). Accumulation factor, in brackets, is expressed as the ratio of the concentration in the organism to the concentration in water.

between 24 h and 48 h of continuous exposure and changed little after an additional exposure (up to 120 h).

Residues in crustaceans after a 24-h exposure to ^{14}C-Bayer 2353 ranged from 4 μg/kg in glass shrimp and crayfish to 80 μg/kg in scuds. Concentrations of residues in scuds declined by 50% within 8 h and by 90% after 48 h in clean flowing water. Aquatic insects exposed continuously accumulated residues in 24 h that ranged from 8 μg/kg for damselfly nymphs to 87 μg/kg for midge larvae. Midge larvae eliminated 50% of the residues in about 11 h and 90% in 48 h.

Discussion

Bayer 73 and TFM are often applied in combination as a single application in tributaries of the Great Lakes to control larval lampreys. Because of costs and the efficacy of the compounds, continuous application to streams never exceeds 12 h (Applegate and King 1962). Since nontarget organisms would generally be exposed to Bayer 73 for only a short time, the acute toxicities would seemingly be most important in assessing the likelihood of mortalities of aquatic invertebrates within the area of a lampricide application; however, some of the chemical may be adsorbed and retained by bottom sediments (Strufe and Gönnert 1962), and benthic organisms could be exposed for a longer time than the duration of the lampricide treatment. Results of our midge emergence studies indicate that there was no effect on emergence of midges at a concentration of 0.32 mg/l, a concentration double that normally applied in sea lamprey control (Howell et al. 1964).

The low accumulation and rapid elimination rate of radioactive residues in scuds and midge larvae suggest that Bayer 73 would not accumulate through the food chain to upper-level consumers. Techniques for determination of degradation products of Bayer 2353 in invertebrates are not well defined, but it is assumed that the loss of radioactivity was due to excretion and degradation of the parent compound.

Field observations and laboratory tests have shown that Bayer 73 is less toxic to most aquatic invertebrates than it is to sea lampreys. Smith (1967) reported that the granular formulation applied in a stream at about three times the normal lampricide application rate had little effect on benthic invertebrates. Kawatski (1973) found that an ostracod *(Cypretta kawatai)* would not be adversely affected at concentrations used to control sea lampreys. Daphnids appear to be more sensitive than most other aquatic invertebrates; consequently, a reduction in population could occur during a lamprey treatment. Daphnid populations could also be reduced if a concentration of 0.1 mg/l was maintained for 21 days. This duration seems highly improbable, since use exposures do not exceed 12 h.

Meyer and Howell (1975) indicated that if a theoretical concentration of 0.11 mg/l were applied to the bottom 5 cm of standing water, the concentration would be sufficiently diluted at 10.7 cm above the bottom to be harmless to trout. Inasmuch as trout are more sensitive to Bayer 73 (Marking and Hogan 1967) than are most of the crustaceans and aquatic insects that have been exposed, concentrations used for lamprey control should have little effect on most arthropods. Moreover, a mixture (98:2 ratio) of TFM and Bayer 73 was more toxic to larval sea lampreys than to nontarget fish in comparable laboratory toxicity tests (Bills and Marking 1976).

The results from this study and others indicate that if standard lamprey control procedures are followed and a Bayer 73 concentration of 0.1 mg/l is not exceeded for more than 24 h, no adverse effect on most aquatic invertebrates should occur. However, data derived from controlled laboratory experiments can serve as guidelines for field applications only after differences in water quality, species of organisms, methods of application, and toxicant formulation have been carefully considered.

Conclusions

1. Invertebrates with a soft integument were more susceptible to Bayer 73 than were those with a chitinized exoskeleton.
2. Daphnid reproduction was significantly reduced ($P<0.01$) in Bayer 73 concentrations of 0.056, 0.10, and 0.18 mg/l.
3. Emergence of midges was significantly reduced ($P<0.01$) in Bayer 73 concentrations of 0.56, 1.0 and 1.8 mg/l.
4. Equilibria of ^{14}C-Bayer 2353 residues were attained in most invertebrates in 24 h, when total body residues (wet weight of whole organism) ranged from 4 to 80 times the water concentration.
5. Elimination of radioactive residues in scuds and midge larvae was rapid: 90% of the accumulated residues were lost within 48 h after the organisms were transferred to clean flowing water.
6. Concentrations of Bayer 73 that are effective in controlling lamprey should have little adverse effect on most aquatic invertebrates.

References

Applegate, V. C., and E. L. King, Jr. 1962. Comparative toxicity of 3-trifluormethyl-4-nitrophenol (TFM) to larval lampreys and eleven species of fishes. Trans. Am. Fish. Soc. 91(4):342-345.

Biever, K. D. 1965. A rearing technique for the colonization of chironomid midges. Ann. Entomol. Soc. Am. 58(2):135-136.

Bills, T.D., and L. L. Marking. 1976. Toxicity of 3-trifluoromethyl-4-nitrophenol (TFM), 2',5-dichloro-4'-nitrosalicylanilide (Bayer 73), and a 98:2 mixture to fingerlings of seven fish species and to eggs and fry of coho salmon. U.S. Fish Wildl. Serv. Invest. Fish Control 69. 11 pp.

Brynildson, C. 1970. Results of chemical fish eradication of Cox Hollow Lake, Iowa County. Wis. Dep. Nat. Resour., Bur. Fish Manage., Manage. Rep. 31. 8 pp.

Chandler, J. H., Jr., and L. L. Marking. 1975. Toxicity of the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) to selected aquatic invertebrates and frog larvae. U.S. Fish Wildl. Serv. Invest. Fish Control 62. 7 pp.

Chandler, J. H., Jr., H. O. Sanders, and D. F. Walsh. 1974. An improved chemical delivery apparatus for use in intermittent-flow bioassays. Bull. Environ. Contam. Toxicol. 12(1):123-128.

Chemagro Corporation. 1970. Bayluscide, molluscicide. Chemagro Corp., Kansas City, Missouri. 6 pp.

Committee on Methods for Toxicity Tests with Aquatic Organisms. 1975. Methods for acute toxicity tests with fish, macroinvertebrates and amphibians. Ecol. Res. Series No. EPA-660/3-75-009. Environ. Prot. Agency, Washington, D.C. 61 pp.

Fremling, C. R. 1975. Acute toxicity of the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) to nymphs of mayflies (*Hexangenia* sp.). U.S. Fish Wildl. Serv. Invest. Fish Control 58. 7 pp.

Gillet, J., and P. Bruaux. 1962. Laboratory and field testing of Bayluscide® ("Bayer 73"). Pflanzenschutz-Nachr.-Bayer 15(1):70-74.

Gönnert, Rudolf. 1962. Bayluscide® a new compound for controlling medically important freshwater snails. Pflanzenschutz-Nachr.-Bayer 15(1):4-25.

Hamilton, S. E. 1974. A review of the literature on the use of Bayluscide in fisheries. U.S. Fish. Wildl. Serv. Lit. Rev. 74-02. Natl. Tech. Inf. Serv. No. PB-235 441/AS. 60 pp.

Howell, J. H., E. L. King, Jr., A. J. Smith, and L. H. Hanson. 1964. Synergism of 5,2'-dichloro-4'-nitro-salicylanilide and 3-trifluormethyl-4-nitrophenol in a selective lamprey larvicide. Great Lakes Fish. Comm., Tech. Rep. 8. 21 pp.

Hunn, J. B. 1973. Registration-oriented research on lampricides in 1972. Pages 70-73 *in* Great Lakes Fishery Commission annual report for the year 1972.

Johnson, B. T., C. R. Saunders, and H. O. Sanders. 1971. Biological magnification and degradation of DDT and aldrin by freshwater invertebrates. J. Fish. Res. Board Can. 28:705-709.

Johnson, B. T., and R. A. Schoettger. 1975. A biological model for estimating the uptake, transfer, and degradation of xenobiotics in an aquatic food chain. Pages 26906-26909 *in* U.S. Environmental Protection Agency, Guidelines for Registering Pesticides in United States. Federal Register, Washington, D.C., Vol. 40, No. 123.

Kawatski, J. A. 1973. Acute toxicities of antimycin A, Bayer 73, and TFM to the ostracod *Cypretta kawatai*. Trans. Am. Fish. Soc. 102(4):829-831.

Kawatski, J. A., M. M. Ledvina, and C. R. Hansen, Jr. 1975. Acute toxicity of 3-trifluoromethyl-4-nitrophenol (TFM) and 2',5-dichloro-4'-nitrosalicylanilide (Bayer 73) to larvae of the midge *Chironomus tentans*. U.S. Fish Wildl. Serv. Invest. Fish Control 57. 7 pp.

Litchfield, J. T., Jr., and F. Wilcoxon. 1949. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther. 96(2):99-113.

Marking, L. L., and J. W. Hogan. 1967. Toxicity of Bayer 73 to fish. U.S. Fish. Wildl. Serv. Invest. Fish Control 19 (Resour. Publ. 36). 13 pp.

Meyer, F. P., and J. H. Howell. 1975. Biological studies on the sea lamprey, 1973. Pages 61-65 *in* Great Lakes Fishery Commission annual report for the year 1973.

Mount, D. L., and W. A. Brungs. 1967. A device for continuous treatment of fish in holding chambers. Water Res. 1:21-29.

Mount, D. I., and C. E. Stephan. 1967. A method for establishing acceptable toxicant limits for fish—malathion and the butoxyethanol ester of 2,4-D. Trans. Am. Fish. Soc. 96(2):185-193.

Sanders, H. O., and O. B. Cope. 1968. The relative toxicities of several pesticides to naiads of three species of stoneflies. Limnol. Oceanogr. 13(1):112-117.

Sanders, H. O., and D. F. Walsh. 1975. Toxicity and residue dynamics of the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) in aquatic invertebrates. U.S. Fish Wildl. Serv. Invest. Fish Control 59. 9 pp.

Smith, A. J. 1967. The effect of the lamprey larvicide, 3-trifluoromethyl-4-nitrophenol, on selected aquatic invertebrates. Trans. Am. Fish. Soc. 96(4):410-413.

Snedecor, G. W., and W. C. Cochran. 1974. Statistical methods. Iowa State Univ. Press, Ames. 327 pp.

Sprague, J. B. 1969. Measurement of pollutant toxicity to fish-I. Bioassay methods for acute toxicity. Water Res. 3:793-821.

Strufe, R., and R. Gönnert. 1962. Comparative studies on the influence of environmental factors upon the efficiency of Bayluscide. Pflanzenschutz-Nachr.-Bayer 15:50-70.

Wild, H., and D. S. Mitchell. 1970. The effect of Bayluscide on the water fern *Salvinia aurticulata* and other aquatic plants. Pflanzenschutz-Nachr.-Bayer 23(2):105-110.

Accumulation, Elimination, and Biotransformation of the Lampricide 2',5-Dichloro-4'-nitrosalicylanilide by *Chironomus tentans*[1]

by

Joseph A. Kawatski and Ann E. Zittel
Department of Biology, Viterbo College
La Crosse, Wisconsin 54601

Abstract

When exposed to sublethal concentrations of ^{14}C-2',5-dichloro-4'-nitrosalicylanilide (Bayer 2353), either alone or in combination with 3-trifluoromethyl-4-nitrophenol (TFM), larvae of the midge *Chironomus tentans* accumlated ^{14}C residue rapidly. Uptake was related directly to amount of toxicant present in exposure water and to water hardness. Residues of ^{14}C-Bayer 2353 did not attain a stable uptake equilibrium but were rapidly excreted, both during continuous exposure and during postexposure periods in toxicant free water. Chironomids biotrans formed ^{14}C-Bayer 2353 to ^{14}C-chlorosalicylic acid and an unidentified ^{14}C metabolite.

In 1958, salicylanilides, particularly 2',5-dichloro-4'-nitrosalicylanilide (Bayer 2353), were discovered to be potent molluscicides (Gönnert 1962). After further investigation, the U.S. Fish and Wildlife Service and the Canadian Department of Environment began in 1966 to use a 5% granular formulation of Bayer 73 (2-aminoethanol salt of Bayer 2353) to control the sea lamprey, *Petromyzon marinus* (Hamilton 1974a, 1974b). Mixtures of Bayer 73 and 3-trifluoromethyl-4-nitrophenol (TFM) in a 2:98 ratio (by weight) have also been used because the addition of small amounts of Bayer 73 substantially reduces the amount of TFM needed for effective treatment of populations of larval lampreys (Howell et al. 1964).

The toxicity of Bayer 73 to nontarget invertebrates varies widely. Bayer 73, at the concentrations used for lamprey control, does not appear to affect many hard-shelled aquatic invertebrates (Rye 1972; Cumming and Dawson 1973; Fish-Pesticide Research Laboratory 1973; Hunn 1973; and Sanders 1973), but some soft-bodied invertebrates are susceptible (Rye 1972). Additional information regarding the dynamics of the toxicant and its residues in nontarget invertebrates is required to satisfy regulatory requirements of the U.S. Environmental Protection Agency.

The present study was designed to determine the rates of accumulation and elimination of Bayer 2353 by larvae of the aquatic midge *Chironomus tentans* (Diptera; Chironomidae) during short-term sublethal exposures. In addition, we investigated the ability of the organism to metabolize the toxicant.

Materials and Methods

Radioactive Bayer 2353 (2',5-dichloro-4'-nitrosalicylanilide) was synthesized with a uniformly labeled ^{14}C-chlorosalicylic acid ring (10mCi/mM, American Radiochemical Corporation, Sanford, Florida). Nonradioactive Bayer 73 (2-aminoethanol salt of 2'-5-dichloro-4'-nitrosalicylanilide, 70% wettable powder) was obtained from the Chemagro Corporation, Kansas City, Kansas. TFM (95.7% 3-trifluoromethyl-4-nitrophenol) was used in a 98:2 (by weight) combination with Bayer 2353; this mixture is designated as TFM-2B. Reconstituted water was prepared as described by Marking (1970) to effect three levels of hardness (mg/l as $CaCO_3$) and (in parentheses) pH: soft, 40-48 (7.2-7.6); hard, 160-180 (7.6-8.0); and very hard, 280-320 (8.0-8.4). These materials were supplied by the Fish Control Laboratory, La Crosse, Wisconsin. All other reagents were of analytical grade unless otherwise specified.

Stock cultures of *Chironomus tentans,* a widely distributed midge whose benthic larva is an important fish food organism, were maintained in continuous laboratory culture in soft water at 21 ± 2 C according to the procedure of Kawatski et al. (1975). Only fourth instar larvae were used in the experiments. Before exposure to the toxicant, the

[1]This study was funded by Contract 14-16-0008-807, U.S. Fish and Wildlife Service.

animals were removed from the rearing system and acclimated in 900 ml of the toxicant free test water at 20 ± 0.5 C for 24 h.

For accumulation studies, larvae were exposed to ^{14}C-Bayer 2353 in Pyrex beakers containing 900 ml of water (60-80 larvae per beaker). Before a sublethal concentration of the toxicant (54-108 μg/l) was added, samples of larvae and test water were withdrawn for background radiation determinations. The toxicant was added in an acetone-water (1:1) solution; the acetone concentration in test water never exceeded 1 ml/l. Triplicate samples of larvae and test water were employed throughout the study.

Immediately after addition of the toxicant, 0.5-ml samples of the exposure water were collected. The rate of toxicant accumulation by larvae was determined by sampling animals and water at 2-h intervals during the first 8 h of exposure, and after 12, 24, 48, 72, and 96 h of exposure. Three larvae per sample were withdrawn from each exposure vessel, placed on an absorbent towel, blotted dry, and weighed. Dry weights were calculated on the basis of a determined dry weight correction factor. Each sample was then transferred to a scintillation vial where 0.5 ml NCS tissue digestor (Amersham/Searle Corp.) was added. The vials were held at room temperature for 2 h before the addition of 10 ml of scintillation cocktail (4 g of 2,4-diphenyloxozole, 0.10 g of 1,4-bis [2(5-phenyloxozolyl)] benzene, 250 ml of Triton X-100, and 750 ml of toluene). The vials were shaken vigorously and then refrigerated until radioactivity of the contents was determined.

In elimination studies, the larvae were transferred from the exposure vessels to 900 ml of toxicant free water after 24 h of exposure to the toxicant. Samples of larvae and water were withdrawn at 2-h intervals and prepared for radioactivity determinations by the same counting procedure as used in the accumulation studies.

Biotransformation studies were conducted by the following procedures. About 100 larvae were exposed to a sublethal concentration of ^{14}C-Bayer 2353 (108 μg/l). Thirty larvae were withdrawn at 12-h intervals over a 36-h period. The larvae were blotted dry, transferred to a grinding vessel, and homogenized with a motor-driven Teflon pestle. We added small amounts of double distilled water, and then subjected the homogenate to ultransonic vibrations, using the intermediate tip of a Sonic 300 Dismembrator (Artex Systems Corporation) at 50% of full power for 10 min.

The sonicated homogenate was spotted directly on precoated thin layer plates purchased from Brinkman Instrument Company (Polygram SIL N-HR, 0.2 mm) and Eastman Kodak Company (6061 Silica Gel, 0.1 mm). The developing systems were: methanol/chloroform/ammonium hydroxide (50/25/2.5,vol/vol/vol; J. J. Lech, personal communication) and methanol/chloroform/acetic acid (16/8/1,vol/vol/vol). For visualization of the separation, we superimposed a mixture of nonradioactive Bayer 73 and chlorosalicylic acid on the spotted sonicate. We vertically sectioned developed plates from 0.5 cm below to 10 cm above the spotting points, and by using the standards as horizontal reference points, cut the vertical sections into several pieces (Kawatski and McDonald 1974). The pieces were placed in individual counting vials with 10 ml of scintillation cocktail, and their radioactivity was determined.

The radioactivity in samples was measured with a Nuclear Chicago Mark II scintillation spectrometer. Observed counts per minute were converted to disintegrations per minute (DPM) with the channels ratio-external standard method of quench correction. For accumulation and elimination studies, DPM's were converted to micrograms of toxicant (in terms of the weight of the parent Bayer 2353 molecule) and expressed in terms of the weight or volume of the sample. In biotransformation studies, the DPM's were summed for each vertical section of the thin layer plate and the percentages of the total contributed by individual horizontal sections were determined.

Results

The magnitude of ^{14}C-Bayer 2353 accumulation from water by *Chironomus tentans* during continuous sublethal exposure was in part a function of the Bayer 2353 concentrations in exposure water. The initial rate of uptake was related both to toxicant concentration and to hardness of exposure water (Fig. 1). For example, when animals were exposed to 63 μg/l of Bayer 2353, uptake rates during the first 12 h were 12.8, 11.9, and 9.6 μg/g per hour in soft, hard, and very hard water; when the exposure concentration was 108 μg/l, the initial uptake rates were 46.7, 42.0, and 31.3 μg/g per hour, respectively.

In nearly all uptake experiments where test animals were exposed continuously to Bayer 2353 for up to 96 h, maximum accumulation occurred during the first 24 h, and usually within 12 h. Residue accumulation did not plateau; rather, the elimination rates thereafter exceeded the uptake rates.

When chironomids that had been exposed to Bayer 2353 were placed in toxicant free water, ^{14}C residues continued to be excreted very rapidly, and the rate of excretion was directly proportional to the toxicant concentration during exposure and to the maximum amount of Bayer 2353 accumulated during the exposure (Fig. 2). Biological half-lives (time required

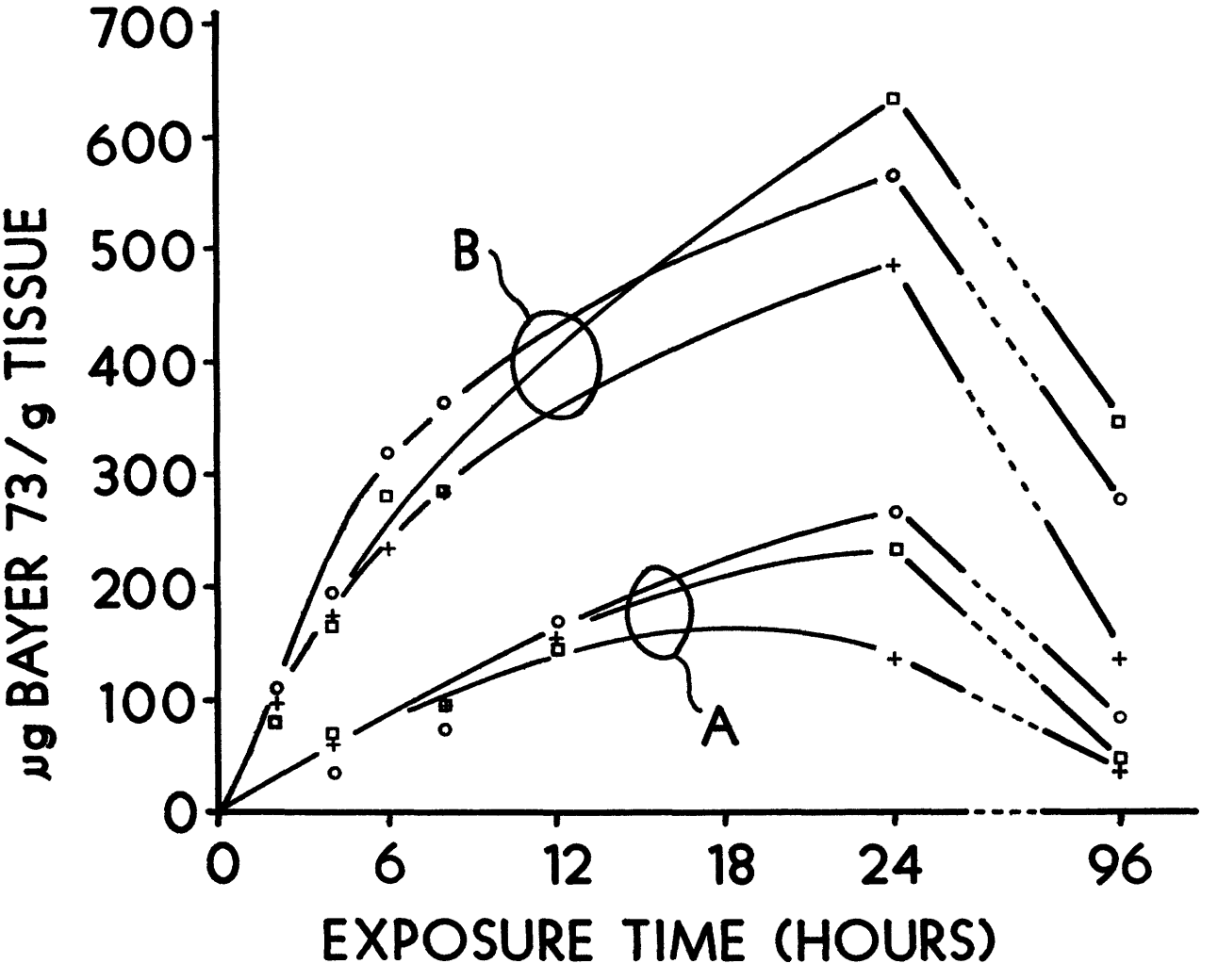

Fig. 1. Uptake of ^{14}C residue ($\mu g/g$ dry wt) by *Chironomus tentans* larvae from water of three hardnesses (0, soft; □, hard; +, very hard) that initially contained 63(A) or 108(B) $\mu g/l$ of ^{14}C-Bayer 2353.

for loss of one-half of the accumulated material) of the residue varied from 3.5 to 25.4 h (Table 1), and the rate of elimination was directly proportional to the maximum amount of residue accumulated during the exposure period (Fig. 3).

Uptake of Bayer 2353 by chironomids exposed to TFM-2B was no different from uptake of Bayer 2353 from test water containing only Bayer 2353 (Table 2). When chironomids were exposed to 2.3 $\mu g/l$ of Bayer 2353 and to TFM-2B (112 $\mu g/l$ of TFM; 2.3 $\mu g/l$ of Bayer 2353), they accumulated ^{14}C residue at the rate of 0.59 $\mu g/g$ per hour during the initial 12 h; thereafter, residue was eliminated even during continuous exposure.

When various substrates (paper, sand, and silt) were placed in exposure vessels, Bayer 2353 concentrations in the water decreased with time at a faster rate than when substrates were absent. Presumably because of the reduction in toxicant concentration, chironomids in the vessels containing substrates absorbed Bayer 2353 residue less rapidly than did control animals in substrate free systems (Table 3).

Chironomids that absorbed ^{14}C-Bayer 2353 were able to cleave the ^{14}C-Bayer 2353 molecule, as evidenced by the recovery of ^{14}C labeled chlorosalicylic acid (Table 4). As exposure continued beyond 8 h, a decreasing percentage of the accumulated residue in chironomid tissue was chlorosalicylic acid and an increasing percentage was Bayer 2353, suggesting that chlorosalicylic acid was being excreted. One other ^{14}C metabolite observed, more polar than either chlorosalicylic acid or Bayer 2353, was probably acidic. A third ^{14}C-labeled component appeared at all exposure times; it did not migrate in

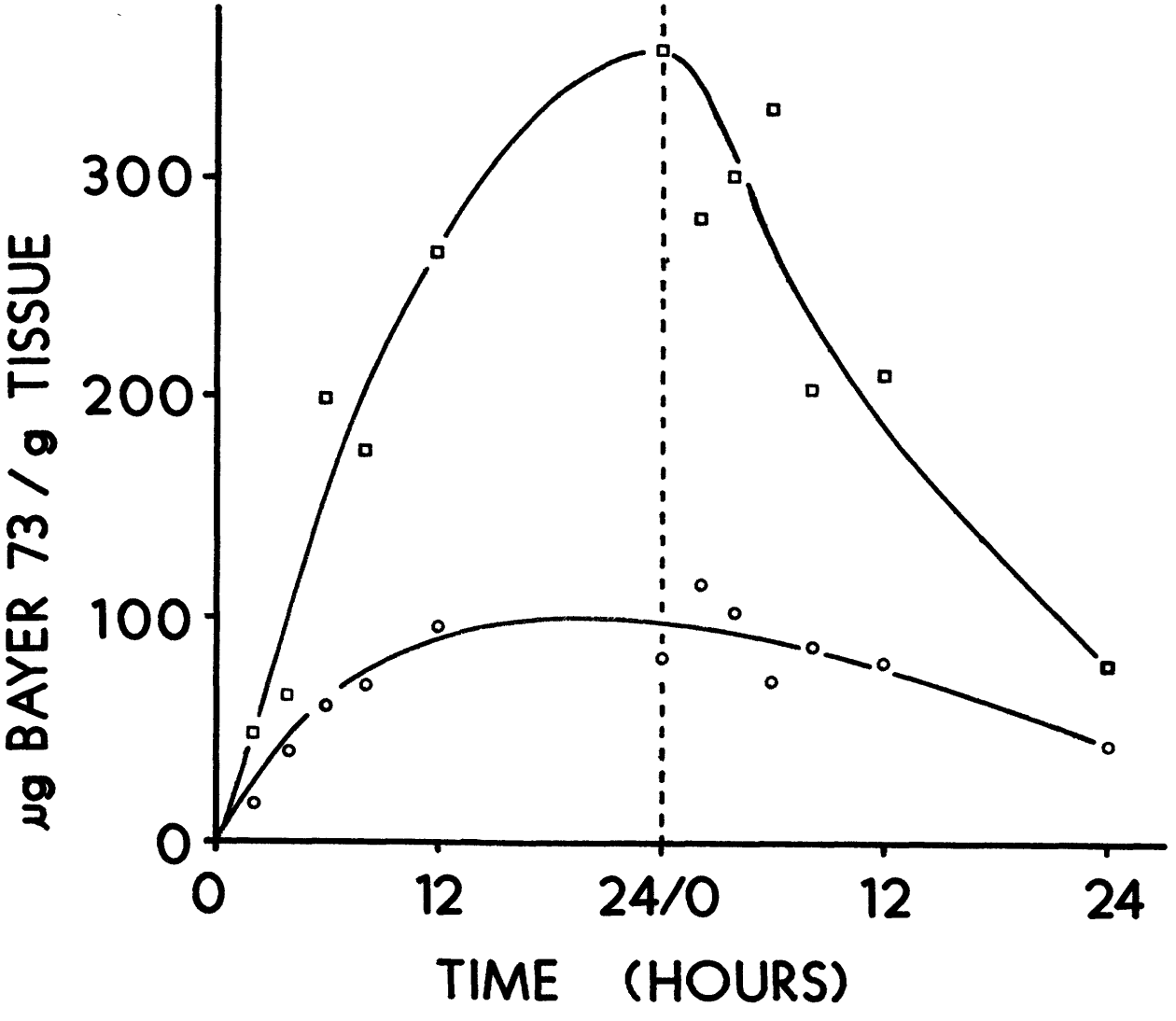

Fig. 2. Uptake of ^{14}C residue ($\mu g/g$ dry wt) by *Chironomus tentans* larvae from soft water that initially contained 54 (0) or 162 (□) $\mu g/l$ of ^{14}C-Bayer 2353, and elimination of residue during a 24-h postexposure period in toxicant free water.

our thin layer chromatographic systems, however, and was not present in the ^{14}C-Bayer 2353 standard.

Discussion

The manner of uptake and elimination of Bayer 2353 by *Chironomus tentans* helps to explain the toxic effects of Bayer 73 observed by Kawatski et al. (1975). In 8-h exposures, Bayer 73 was more toxic in soft water than in hard water, but in longer exposures (24-96 h) the differences in toxicity related to water hardness became progressively less, and were statistically nonsignificant. The uptake pattern for ^{14}C-Bayer 2353 is consistent with the toxicity data from previous static tests. During the first 8 h of exposure, the rate of ^{14}C-Bayer 2353 uptake decreased with increasing water hardness, but after about 12 h of exposure, residue levels did not vary significantly in relation to hardness. Furthermore, after 12 h of exposure and during continuous exposure, ^{14}C residue was eliminated regardless of water hardness. The toxicological result of this excretion is that 48-, 72-, and 96-h LC_{50} values do not differ significantly at any given water hardness (Kawatski et al. 1975).

Accumulation of ^{14}C-Bayer 73 is independent of TFM uptake; i.e., Bayer 2353 absorption is identical from solutions of either TFM-2B or Bayer 2353 alone.

Table 1. *Accumulation of ^{14}C residue ($\mu g/g$ dry wt) by* Chironomus tentans *larvae at 20±1 C under various conditions of exposure to ^{14}C-Bayer 2353, elimination of ^{14}C residue during postexposure in toxicant free water of differing hardness and pH, and biological half-life of accumulated ^{14}C residue*[a].

Initial exposure concn. of Bayer 2353 ($\mu g/l$)[a]	Exposure water hardness[b]	Length of exposure (h)	Total ^{14}C residue accumulated ($\mu g/g$)[c]	Postexposure water hardness[b]	Biological half-life of ^{14}C residue (h)
Experiment 1					
54	S	24	79	S	25.4
162	S	24	356	S	12.6
Experiment 2					
108	H	8	171	H	12.0
108	H	12	164	H	10.7
Experiment 3					
108	S	24	567	S	15.2
108	S	24	567	H	5.0
108	S	24	567	VH	3.7
Experiment 4					
108	H	24	634	S	3.5
108	H	24	634	H	3.5
108	H	24	634	VH	3.5
Experiment 5					
108	VH	24	488	S	5.2
108	VH	24	488	H	5.2
108	VH	24	488	VH	4.2

[a] Results of simultaneous tests are grouped as experiments.

[b] S = soft (hardness, 40-48 mg/l as $CaCO_3$; pH, 7.2-7.6); H = hard (160-180 mg/l; pH, 7.6-8.0); and VH = very hard (280-320 mg/l; pH, 8.0-8.4).

[c] Expressed in terms of the weight and activity of the parent Bayer 2353 molecule.

Kawatski and Bittner (1975) found that accumulation of TFM was likewise independent of Bayer 73 uptake. Therefore the slight synergistic toxicity of TFM and Bayer 73 is not due to potentiated uptake at absorptive surfaces but must be due to other interactions.

The ability of chironomids to metabolize ^{14}C-Bayer 2353 to form at least two compounds (chlorosalicylic acid and a more polar material) probably accounts in part for the rapid excretory rate. The unidentified polar material is probably a conjugated form of either Bayer 2353 itself or of chlorosalicylic acid. Salicylates are readily conjugated with glycine and glucuronic acid in higher organisms (Milne 1963), and chironomids also possess such detoxication systems (Kawatski and Bittner 1975). Since only the chlorosalicylic acid ring of the Bayer 2353 was labeled, the chloronitroaniline ring and possible derivatives of it were not recovered or measured.

The ^{14}C material that did not migrate on thin layer plates (nonmigrating material; NMM) cannot with certainty be identified as a Bayer 2353 metabolite, since it may represent an artifact of the analytical method. If Bayer 2353 or its metabolites are trapped within cell fragments which remain immobile, the material identified as NMM may simply be Bayer 2353, chlorosalicylic acid, or the more polar unidentified derivative. Although we attempted to disrupt subcellular components by sonicating the tissue homogenates before performing thin layer chromatography, some salicylates probably remained bound to proteins (a well-known tendency for salicylates). Thus the identity of the NMM remains undetermined.

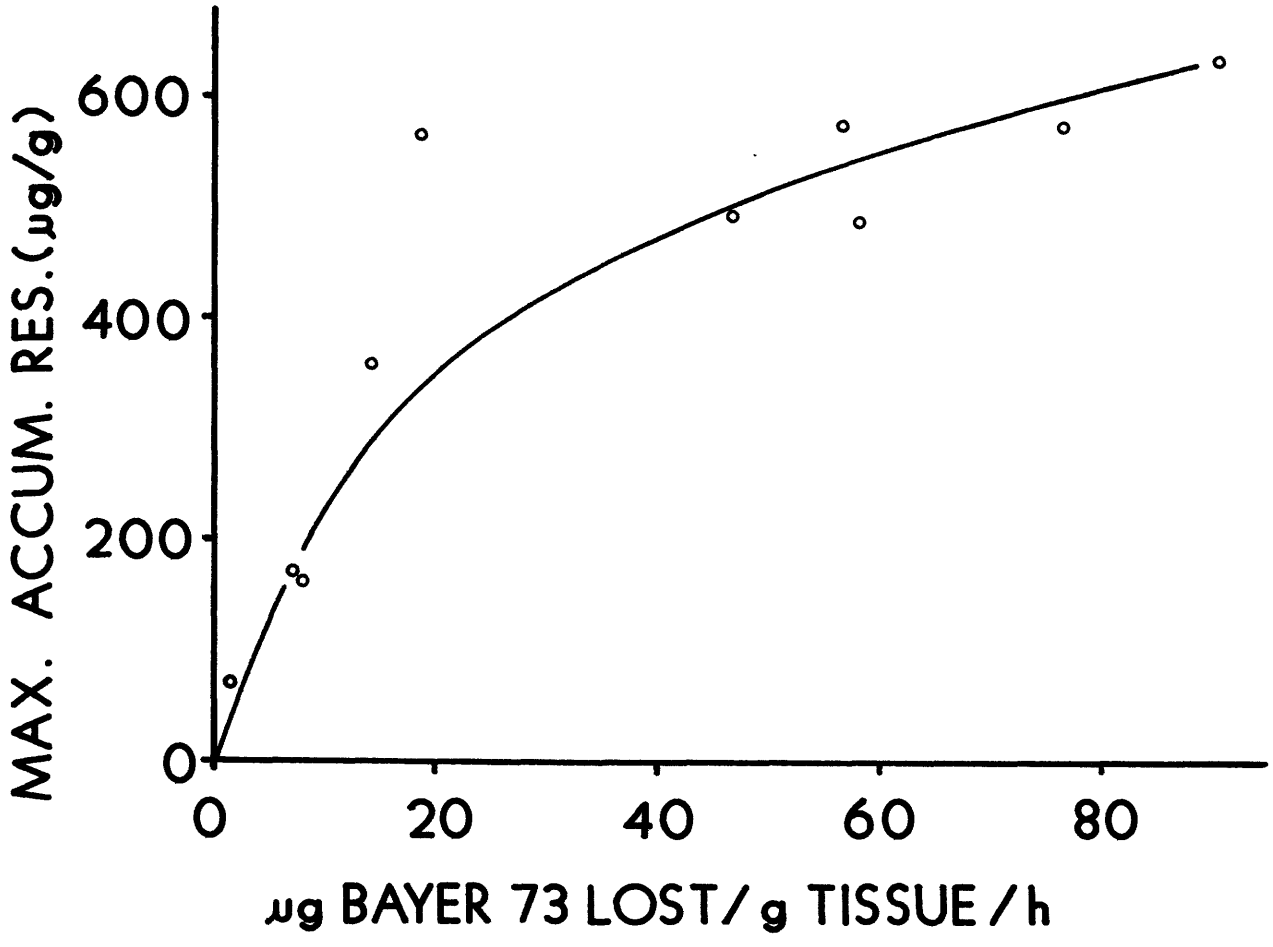

Fig 3. Relation between rate of elimination of accumulated ^{14}C residue and maximum body burden of residue (µg/g dry wt).

Table 2. *Accumulation of ^{14}C residue by* Chironomus tentans *larvae during 96 h of continuous exposure to sublethal concentrations of ^{14}C-Bayer 2353 and TFM-2B[a] in soft water ($CaCO_3$ hardness, 40-48 mg/l; pH, 7.2-7.6) at 20 + 1 C[b].*

Length of exposure (h)	Exposed to Bayer 2353		Exposed to TFM-2B	
	Exposure water (µg/l)	Tissue (µg/g dry wt)	Exposure water (µg/l)	Tissue (µg/g dry wt)
0	2.52 (0.11)	—	2.52 (0.12)	—
2	2.39 (0.23)	1.55 (0.41)	2.29 (0.04)	2.09 (0.15)
4	2.14 (0.24)	2.32 (0.32)	2.21 (0.11)	2.49 (0.35)
8	1.99 (0.15)	4.84 (1.63)	1.98 (0.19)	4.64 (1.40)
12	1.88 (0.05)	6.37 (1.04)	1.97 (0.29)	6.64 (1.38)
24	1.73 (0.13)	3.72 (1.06)	1.74 (0.03)	5.20 (0.60)
96	1.78 (0.12)	1.69 (0.16)	2.13 (0.06)	1.65 (0.12)

[a] TFM-2B designates a 98:2 (by weight) combination of TFM and Bayer 2353.

[b] Standard deviations are shown in parentheses.

Table 3. *Accumulation of ^{14}C residue ($\mu g/g$ dry wt) by* Chironomus tentans *larvae during 36 h of continuous exposure to ^{14}C-Bayer 2353 in soft water ($CaCO_3$ hardness, 40-48 mg/l; pH, 7.2-7.6) at 20 + 1 C containing 5 g of the named substrates in 900 ml of test medium*[a].

Substrate and mesh number (where applicable)	Duration of exposure (h)					Water characteristics at end of exposure			
	4	8	12	24	36	Bayer 2353 ($\mu g/l$)	Hardness (mg/l as $CaCO_3$)	Dissolved oxygen (mg/l)	pH
None	43.3 (8.7)	121.6 (17.8)	99.5 (6.7)	155.6 (105.9)	185.1 (95.2)	67.1 (1.7)	52	7.7	6.8
Paper toweling	34.3 (11.6)	36.1 (1.0)	74.1 (13.9)	71.0 (22.6)	54.3 (13.6)	40.9 (0.8)	53	7.3	6.8
Sand									
30	36.3 (13.0)	42.6 (12.3)	76.5 (15.9)	41.9 (9.1)	34.4 (1.1)	60.5 (1.3)	57	8.3	6.8
80	22.7 (12.0)	23.7 (1.9)	23.8 (2.7)	30.0 (6.9)	24.4 (5.0)	44.8 (0.8)	78	6.0	6.9
200	28.7 (12.5)	59.2 (13.2)	41.2 (3.1)	74.0 (22.6)	51.4 (8.5)	61.7 (3.4)	70	7.3	6.8
Silt									
30	23.3 (18.8)	45.0 (17.4)	44.5 (22.0)	34.3 (8.8)	39.7 (6.4)	44.1 (1.5)	120	5.3	7.0
80	38.2 (15.3)	95.0 (58.6)	50.1 (34.3)	65.4 (56.1)	26.1 (9.2)	26.4 (1.0)	140	5.0	6.8

[a] Initial concentration of ^{14}C-Bayer 2353: 66.0 $\mu g/l$; standard deviations are shown in parentheses.

Table 4. *Percentages of total ^{14}C residue retained by* Chironomus tentans *larvae exposed continuously to ^{14}C-Bayer 2353 in soft water ($CaCO_3$ hardness, 40-48 mg/l; pH, 7.2-7.6) at 20 + 1 C*[a].

Component	Thin layer chromatographic identification R_f[b] (ranges in parentheses)	Exposure period (h)[c] 8	24	36
Bayer 2353	0.65 (0.55-0.70)	20.7 (3.2)	23.8 (2.9)	39.1[d] (13.1)
Chlorosalicylic acid	0.45 (0.30-0.55)	32.5 (4.0)	29.9 (4.3)	23.7[d] (13.0)
Other[b]	0.25 (0.10-0.30)	18.1 (4.3)	15.9 (3.1)	8.8[d] (2.4)
NMM[e]	0.0 (-0.05-0.10)	28.5 (1.7)	29.2 (2.9)	26.2 (3.9)

[a] Initial concentration of ^{14}C-Bayer 2353: 108 μg/l.
[b] R_f's for both acidic and basic thin layer systems; "other" material appeared to migrate slightly behind Bayer 2353 in the basic system.
[c] Standard deviations given in parentheses.
[d] Change between 8 and 36 h statistically significant (P = 0.05).
[e] NMM: nonmigrating material; the amounts of NMM were similar in both basic and acidic thin layer developing systems.

Conclusions

1. Larvae of *Chironomus tentans* accumulated Bayer 2353 rapidly from sublethal exposure concentrations.
2. Accumulation of residue depended in part on water hardness and concentration of the toxicant.
3. Chironomids eliminated residue rapidly, both during continuous exposure and during postexposure periods in toxicant free water.
4. Rates of Bayer 2353 accumulation and elimination were independent of TFM absorption and elimination.
5. *C. tentans* metabolized ^{14}C-Bayer 2353, producing ^{14}C-chlorosalicylic acid and at least one other ^{14}C product.

References

Cumming, K. B., and V. K. Dawson. 1973. Laboratory efficacy of lampricides. Presented at Great Lakes Fishery Commission Interim Meeting, Ann Arbor, Michigan, December 4-5, 1973. 2 pp.

Fish-Pesticide Research Laboratory. 1973. Quarterly progress report July-September 1973. U.S. Fish Wildl. Serv., Fish-Pest. Res. Lab., Columbia, Missouri. 29 pp.

Gönnert, R. 1962. Bayluscide, a new compound for controlling medically important freshwater snails. Pflanzenschutz-Nachr. 15:4-24.

Hamilton, S. 1974a. A review of the literature on the use of Bayluscide in fisheries. U.S. Fish Wildl. Serv. Lit. Rev. 72-02. Natl. Tech. Inf. Serv. No. PB-235 441/AS. 60 pp.

Hamilton, S. 1974b. A review of the literature on the use of TFM-Bayluscide in fisheries. U.S. Fish Wildl. Serv. Lit. Rev. 74-03. Natl. Tech. Inf. Serv. No. PB-235 442/AS. 39 pp.

Howell, J. H., E. L. King, Jr., A. J. Smith, and L. H. Hanson. 1964. Synergism of 5,2'-dichloro-4'-nitro-salicylanilide and 3-trifluormethyl-4-nitrophenol in a selective lamprey larvicide. Great Lakes Fish. Comm., Tech. Rep. 8. 21 pp.

Hunn, J. B. 1973. Registration-oriented research on TFM in 1972. Appendix 22, pages 310-315 *in* Great Lakes Fishery Commission Report of annual meeting, Ottawa, Ontario, June 19-21, 1973.

Kawatski, J. A., and M. A. Bittner. 1975. Uptake, elimination, and biotransformation of the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) by larvae of the aquatic midge *Chironomus tentans*. Toxicology 4:183-194.

Kawatski, J. A., M. M. Ledvina, and C. R. Hansen, Jr. 1975. Acute toxicities of 3-trifluoromethyl-4-nitrophenol (TFM) and 2',5-dichloro-4-nitrosalicylanilide (Bayer 73) to larvae of the midge *Chironomus tentans*. U.S. Fish Wildl. Serv. Invest. Fish Control No. 57. 7 pp.

Kawatski, J. A., and M. J. McDonald. 1974. Effect of 3-trifluoromethyl-4-nitrophenol on *in vivo* tissue respiration of four species of fish with preliminary notes on its *in vitro* biotransformation. Comp. Gen. Pharmacol. 5:67-76.

Marking, L. L. 1970. Juglone (5-hydroxy-1,4-napthoquinone) as a fish toxicant. Trans. Am. Fish. Soc. 99(3):510-514.

Milne, M. D. 1963. The excretion of salicylate and its metabolites. Pages 18-27 *in* Salicylates: an international symposium. J & A Churchill Ltd., London. 310 pp.

Rye, P. R., Jr. 1972. The toxicity of two lamprey larvicides to selected aquatic invertebrates. U.S. Fish Wildl. Serv., Hammond Bay Biological Station, Millersburg, Michigan. 14 pp. (Unpubl. manuscr.)

Sanders, H. O. 1973. Toxicity and accumulation of Bayer 73 in aquatic invertebrates. Presented at Great Lakes Fishery Commission Interim Meeting, Ann Arbor, Michigan, December 4-5, 1973. 1 p.

(Reports 60 through 62 are in one cover.)

60. Toxicity of the Lampricide 3-Trifluoromethyl-4-nitrophenol (TFM) to Nontarget Fish in Static Tests, by L. L. Marking and L. E. Olson. 1975. 27 pp.
61. Toxicity of the Lampricide 3-Trifluoromethyl-4-nitrophenol (TFM) to Nontarget Fish in Flow-Through Tests, by L. L. Marking, T. D. Bills, and J. H. Chandler. 1975. 9 pp.
62. Toxicity of the Lampricide 3-Trifluoromethyl-4-nitrophenol (TFM) to Selected Aquatic Invertebrates and Frog Larvae, by J. H. Chandler and L. L. Marking. 1975. 7 pp.

(Reports 63 through 66 are in one cover.)

63. Laboratory Efficacy of 3-Trifluoromethyl-4-nitrophenol (TFM) as a Lampricide, by V. K. Dawson, K. B. Cumming, and P. A. Gilderhus. 1975. 7 pp.
64. Effects of 3-Trifluoromethyl-4-nitrophenol (TFM) on Developmental Stages of the Sea Lamprey, by G. W. Piavis and J. H. Howell. 1975. 4 pp.
65. Accumulation and Loss of Residues of 3-Trifluoromethyl-4-nitrophenol (TFM) in Fish Muscle Tissue: Laboratory Studies, by J. B. Sills and J. L. Allen. 1975. 5 pp.
66. Residues of 3-Trifluoromethyl-4-nitrophenol (TFM) in a Stream Ecosystem after Treatment for Control of Sea Lampreys, by P. A. Gilderhus, J. B. Sills, and J. L. Allen. 1975. 5 pp.
67. Method for Assessment of Toxicity or Efficacy of Mixtures of Chemicals, by L. L. Marking and V. K. Dawson. 1975. 7 pp.
68. Development and Evaluation of On-site Toxicity Test Procedures for Fishery Investigations, by Ralph M. Burress. 1975. 8 pp.

(Reports 69 and 70 are in one cover.)

69. Toxicity of 3-trifluoromethyl-4-nitrophenol (TFM), 2′,5-dichloro-4′-nitrosalicylanilide (Bayer 73), and a 98:2 Mixture to Fingerlings of Seven Fish Species and to Eggs and Fry of Coho Salmon, by T. D. Bills and L. L. Marking. 1976. 9 pp.
70. The Freshwater Mussel (*Anodonta* sp.) as an Indicator of Environmental Levels of 3-trifluoromethyl-4-nitrophenol (TFM), by A. W. Maki and H. E. Johnson. 1976. 5 pp.
71. Field Tests of Isobornyl Thiocyanoacetate (Thanite) for Live Collection of Fishes, by R. M. Burress, P. A. Gilderhus, and K. B. Cumming. 1976. 13 pp.
72. Toxicity of Rotenone to Fish in Standardized Laboratory Tests, by L. L. Marking and T. D. Bills. 1976. 11 pp.

(Reports 73 through 76 are in one cover.)

73. Formalin: Its Toxicity to Nontarget Aquatic Organisms, Persistence, and Counteraction, by T. D. Bills, L. L. Marking, and J. H. Chandler, Jr. 1977. 7 pp.
74. Chlorine: Its Toxicity to Fish and Detoxification of Antimycin, by L. L. Marking and T. D. Bills. 1977. 6 pp.
75. Malachite Green: Its Toxicity to Aquatic Organisms, Persistence, and Removal with Activated Carbon, by T. D. Bills, L. L. Marking, and J. H. Chandler, Jr. 1977. 6 pp.
76. Toxicity of Furanace® to Fish, Aquatic Invertebrates, and Frog Eggs and Larvae, by L. L. Marking, T. D. Bills, and J. H. Chandler, Jr. 1977. 6 pp.

Fish Control Laboratories
Fish and Wildlife Service
U.S. Department of the Interior
P.O. Box 862
La Crosse, Wisconsin 54601

As the Nation's principal conservation agency, the Department of the Interior has responsibility for most of our nationally owned public lands and natural resources. This includes fostering the wisest use of our land and water resources, protecting our fish and wildlife, preserving the environmental and cultural values of our national parks and historical places, and providing for the enjoyment of life through outdoor recreation. The Department assesses our energy and mineral resources and works to assure that their development is in the best interests of all our people. The Department also has a major responsibility for American Indian reservation communities and for people who live in island territories under U.S. administration.

GPO 837-725

FISH CONTROL LABORATORIES
U.S. FISH AND WILDLIFE SERVICE
P.O. BOX 862
LA CROSSE, WISCONSIN 54601

POSTAGE AND FEES PAID
U.S. DEPARTMENT OF THE INTERIOR
INT 423